

Use of an analytical model to study limitations on net photosynthesis in *Arbutus unedo* under field conditions

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Summary. From field gas-exchange measurements on *Arbutus unedo* growing in Portugal, parameter values necessary to apply an analytical, physiologically based model of C_3 photosynthesis were obtained. The model successfully simulated measured diurnal photosynthetic responses in *Arbutus* during periods without water stress, under both natural and CO_2 -saturating conditions. The model was used to analyze those factors limiting primary productivity during each of the experimental days. Due to a large investment in ribulose biphosphate (RuBP) regeneration capacity, irradiance was rarely limiting, even during cloudy periods, but the limitation imposed by stomatal conductance was quite large, averaging over 30%. The fact that experimental leaves were maintained in a horizontal position is at least partially responsible for these results. Possible other reasons for this apparent excess of RuBP regeneration capacity vis-a-vis RuBP carboxylase-oxygenase concentration are discussed.

Key words: Photosynthesis model – RuBP regeneration – Stomatal limitation – *Arbutus*

Under field conditions, incident irradiance, leaf temperature and internal partial pressure of CO_2 may fluctuate independently, and interactively determine carbon assimilation. The identification of those environmental factors responsible for limiting assimilation at a given time requires an understanding of these interactions. We have found analytical models of whole-leaf photosynthesis useful in identifying limiting factors under field conditions in apricot (Tenhunen et al. 1980a), soybean (Tenhunen et al. 1980b), *Quercus coccifera* (Tenhunen et al. 1985) and sugar maple (Weber et al. 1985a). WHOLEPHOT, the model used in the first two of these studies, is based on the no longer tenable assumption that ribulose biphosphate (RuBP) is always present in saturating amounts (Farquhar and von Caemmerer 1982; Harley 1982). The model utilized in the oak and maple studies contained an empirical description of the carbon dioxide response, but provided a useful framework within which to study seasonal changes in photosynthetic properties (Tenhunen et al. 1984b; Harley et al. 1986). In a series of papers (Farquhar et al. 1980; von Caemmerer and Farquhar 1981; Farquhar and von Caem-

merer 1982) Farquhar and his colleagues developed a physiologically-based model of whole leaf photosynthesis, based on enzyme kinetics, whole-chain electron transport, and the energy requirements of RuBP regeneration. Using parameter values obtained in vitro from isolated chloroplast or enzyme studies, von Caemmerer and Farquhar (1981) were able to generate photosynthetic response curves which realistically simulated the responses of *Phaseolus vulgaris* leaves.

Harley et al. (1985) described ways in which parameters might be determined solely from whole-leaf gas exchange data. Using soybean, they were able to estimate the temperature dependencies of those parameters used to describe carboxylation and oxygenation, and obtain close agreement between measured data and model simulations. The simulations, however, were of those data sets used in the determination of model parameters, and it remained to be demonstrated that the model could simulate an independent data set obtained under a wide range of incident irradiances, leaf temperatures and intercellular partial pressures of CO_2 . Nor was it demonstrated that model parameters could be obtained under field conditions. The research discussed here addresses those issues.

Arbutus unedo is a sclerophyllous evergreen shrub, characteristic of Mediterranean coastal scrub vegetation, and the subject of intensive study in recent years under both field and laboratory conditions, primarily because stomatal conductance and net photosynthesis exhibit strong sensitivity to high temperature and low humidity. Tenhunen et al. (1980c), Tenhunen et al. (1981), Tenhunen et al. (1982), Lange et al. (1982) and Lange et al. (1985) studied midday stomatal closure in *Arbutus*, Burschka et al. (1985) investigated the role of abscisic acid in this phenomenon, and Beyschlag (1984) studied annual primary production and regulation of gas exchange. In an attempt to understand further the environmental factors limiting CO_2 assimilation in *Arbutus*, we obtained in May 1983 leaf photosynthetic responses to temperature and internal partial pressures of CO_2 to enable us to estimate model parameters and their temperature dependencies. Using the model, we then simulated measured diurnal photosynthetic response data obtained under natural conditions. The close correspondence between measured and simulated data, and the utility of the model in elucidating those factors responsible for limiting photosynthesis under varying environmental conditions, indicate that it is possible to utilize a model of consid-

erable complexity in analyzing plant responses under field conditions.

Model theory

The model predicts net CO₂ assimilation rates using as inputs leaf temperature, incident photosynthetically active radiation (PAR) and stomatal conductance (*g*). The partial pressure of CO₂ (*p*(CO₂)) at the site of fixation, also necessary in the calculations, is assumed equal to intercellular CO₂ (*P_i*) (see Farquhar and von Caemmerer (1982) and Sharkey (1985) for discussion) and is determined in an iterative manner, discussed further below. To assess the role of photorespiration, the partial pressure of O₂ (*p*(O₂)), 210 mbar, is also input. For a given set of environmental conditions, therefore, the model allows one to assess the relative limitations provided by each of the environmental inputs. Inasmuch as *P_i* is dependent in part on stomatal conductance, it is also possible to assess the limiting role of stomatal behavior.

For a complete discussion of the theory on which the model is based, refer to Farquhar and von Caemmerer (1982) and Harley et al. (1985). The primary assumption underlying the model is that photosynthesis is controlled at the enzyme RuBP carboxylase-oxygenase and dependent on *p*(CO₂) and *p*(O₂) at the site of fixation, with a potential RuBP limitation imposed via regeneration of the acceptor by the Calvin cycle and/or electron transport and photophosphorylation. Farquhar and von Caemmerer (1982) suggest a further potential limitation due to inorganic phosphate and triose phosphate utilization, an idea developed further by Sharkey (1985) but such a limitation is not included in this study.

Based on the stoichiometry of the integrated photosynthetic and photorespiratory cycles (Farquhar et al. 1980), 0.5 mol of CO₂ is released in the photorespiratory carbon oxidation (PCO) cycle for each mol of O₂ reduced at RuBP carboxylase-oxygenase. If RuBP is not limiting, net assimilation (*A*) may be expressed,

$$A = W_c - 0.5 W_o - R_d \quad (1)$$

where *W_c* and *W_o* are rates of carboxylation and oxygenation, respectively, under conditions of saturating RuBP, and *R_d* is CO₂ release in the light by processes other than the PCO cycle, here termed "day" respiration. Both *W_c* and *W_o* are assumed to obey competitive Michaelis-Menten kinetics, such that

$$W_c = \frac{V_{c_{\max}} C}{C + K_c(1 + O/K_o)} \quad (2a)$$

and

$$W_o = \frac{V_{o_{\max}} O}{O + K_o(1 + C/K_c)} \quad (2b)$$

where *C* and *O* are *p*(CO₂) and *p*(O₂) at the site of fixation, *V_{c_{max}}* and *K_c* are the maximum velocity and Michaelis constant for carboxylation and *V_{o_{max}}* and *K_o* those for oxygenation. Combining Eqs. (1), (2a) and (2b) and following the terminology of Farquhar and von Caemmerer (1982), net assimilation under conditions of RuBP saturation may be expressed

$$A = \frac{V_{c_{\max}}(C - \Gamma'^*)}{C + K_c(1 + O/K_o)} - R_d \quad (3)$$

where Γ'^* is the CO₂ compensation point in the absence of *R_d*, and

$$\Gamma'^* = \frac{0.5 V_{o_{\max}} \cdot K_c \cdot O}{V_{c_{\max}} \cdot K_o}$$

Adopting the assumption of Farquhar et al. (1980) that $V_{o_{\max}} = 0.21 * V_{c_{\max}}$ and expanding Eq. (3)¹,

$$A = \frac{V_{c_{\max}} C K_o - 0.105 V_{c_{\max}} O K_c}{K_c O + K_c K_o + K_o C} - R_d \quad (4)$$

Since each carboxylation and each oxygenation utilizes a single molecule of RuBP, the sum of *W_c* and *W_o* represents the maximum potential rate of RuBP utilization. If RuBP concentration is limiting, each molecule of RuBP regenerated in the Calvin cycle is utilized immediately. Thus, the measured rate of assimilation under CO₂-saturating conditions, here designated *P_m*, reflects the maximum potential rate of RuBP regeneration, and the ratio *P_m*/(*W_c* + *W_o*) compares potential RuBP regeneration with potential RuBP utilization. If the ratio is greater than one, potential RuBP regeneration exceeds potential utilization, RuBP is non-limiting, and Eq. (4) applies. If this ratio is less than one, however, RuBP must limit the reaction. The extent of this limitation is closely approximated by the ratio *P_m*/(*W_c* + *W_o*) (Harley 1982). This information may be summarized in the following expression,

$$A = \frac{V_{c_{\max}} C K_o - 0.105 V_{c_{\max}} O K_c}{K_c O + K_c K_o + K_o C} \cdot \min \left\{ 1, \frac{P_m}{W_c + W_o} \right\} - R_d \quad (5)$$

where "min { }" denotes "minimum of".

The CO₂-saturated rate of photosynthesis at a given leaf temperature, *P_m*, is a function of PAR. As discussed in Tenhunen et al. (1976), this dependency may be described according to an empirical equation (Smith 1937),

$$P_m = \frac{\alpha I}{\left(1 + \frac{I^2}{P_{m1}^2} \right)^{\frac{1}{2}}} \quad (6)$$

where *I* is incident PAR, α is the maximum efficiency of incident light energy conversion, i.e., the initial slope of the light response, and *P_{m1}* is the assimilation rate at saturating PAR and saturating *p*(CO₂). The temperature dependency of *P_{m1}* may be described using the equation of Johnson et al. (1942),

$$P_{m1} = \frac{T_K \cdot e^{(c - \Delta H_a / RT_K)}}{1 + e^{(\Delta S T_K - \Delta H_d) / (RT_K)}} \quad (7)$$

where *T_K* is leaf temperature in °K, *c* is a constant, ΔH_a and ΔH_d are activation energies of the reaction and the denaturation equilibrium, respectively, ΔS is the entropy of the denaturation equilibrium and *R* is the gas constant (8.31 J K⁻¹ mol⁻¹).

1 Brooks and Farquhar (1985) have recently described an elegant way of measuring Γ'^* , allowing one to utilize Eq. (3) and obviating the need to adopt the problematic assumption that $V_{o_{\max}} = 0.21 * V_{c_{\max}}$ used in this analysis. Analysis using Γ'^* would lead to somewhat different parameter values than those obtained here, but would not significantly affect fit to the data or our general conclusions

The temperature dependency of "day" respiration, R_d , is described by an Arrhenius function,

$$R_d = e^{(d - E/RT\kappa)} \quad (8)$$

where d is a constant and E is the activation energy.

Methods

All gas exchange measurements were conducted on single mature shoots of *Arbutus unedo* growing in a natural evergreen macchia at the Research Station Quinta São Pedro, Sobreda, Portugal. All data reported were obtained during mid-May 1983, prior to the onset of summer drought. Predawn water potentials averaged -4.5 bars and midday minima -20.5 bars during the study, indicating that water stress was not a significant factor. A shoot with several leaves was enclosed in a gas-exchange cuvette (Walz Meß- und Regeltechnik, Effeltrich, FRG) which was part of a mobile laboratory. The methods used for measurements of gas-exchange are described in detail by Tenhunen et al. (1984a). Incoming dew point was set using a water trap and dew point within the leaf cuvette was controlled and transpiration measured by trapping transpired water in a closed loop bypass. Cuvette air temperature was controlled using Peltier heat exchangers. The $p(\text{CO}_2)$ incoming to the cuvette was constantly monitored with an infrared gas analyzer in absolute mode (BINOS, Leybold-Heraeus, Hanau, FRG), and CO_2 exchange by the leaf was measured using a second gas analyzer in differential mode. The leaves were restrained in a horizontal position by a monofilament mesh, leaf temperature was measured using 36-gauge copper/constantan thermocouples appressed to the abaxial side of a leaf, and incident PAR was sensed using a quantum sensor (LI-COR, Lincoln, NB, USA) within the cuvette. Environmental inputs were recorded at four-minute intervals, as were net photosynthesis, transpiration rates and leaf conductance, all calculated as described in Tenhunen et al. (1984a) and based on equations of von Caemmerer and Farquhar (1981) which incorporate corrections for water vapor in the gas stream. All rates are expressed on a projected leaf area basis, but conversions may be made using the specific leaf weight of 0.17 g cm^{-2} . Leaf water potentials were measured using a Scholander pressure chamber.

During experiments to obtain diurnal gas exchange responses under natural conditions, ambient air, buffered against slight fluctuations, was routed to the cuvette, and cuvette temperature and dew point were controlled to track ambient conditions. When necessary to obtain shoot responses to single variables, both cuvette temperature and dew point could be controlled to a desired value. For those experiments where $p(\text{CO}_2)$ was varied, pure CO_2 and CO_2 -free air were mixed using mass flow controllers (Tylan Corp., Carson, CA, USA). Finally, for experiments in which we sought to eliminate photorespiration by reducing $p(\text{O}_2)$ to approx. 10 mbar, CO_2 and N_2 were mixed using mass flow controllers to obtain the desired $p(\text{CO}_2)$, and a small amount of air from which CO_2 had been scrubbed was added to the mixture using a diaphragm pump. The $p(\text{O}_2)$ was measured using an oxygen electrode.

The analysis described in this paper requires fitting complex functions to measured data. All such curve fitting has been performed by non-linear least-squares regression analysis, using a statistical program, BMDX85 (Dixon 1969),

provided by the University of Michigan Statistical Laboratory (Ann Arbor, MI, USA).

Results

This model contains a large number of parameters and the task of obtaining values and temperature dependencies for each under field conditions may seem unrealistic. Farquhar and von Caemmerer (1982) suggest, however, that photosynthetic variation within C_3 species may be largely explained by changes in two key parameters, $V_{c_{\max}}$ and their J_{\max} (proportional to P_{m1}), to which we would add a third, R_d . $V_{c_{\max}}$ is proportional to the amount of RuBP carboxylase and P_{m1} to the amount of electron transport components and Calvin cycle enzymes, all of which may vary greatly within and between species (see Farquhar and von Caemmerer 1982). Similarly, mitochondrial respiration, and presumably "day" respiration, show considerable variation. Other model parameters may be expected to show less variation. For example, α is proportional to quantum efficiency, which is relatively constant for all C_3 plants (Ehleringer and Björkman 1977), and will vary in the context of this model only as leaf absorption changes. Similarly, K_m 's represent intrinsic properties of the enzyme, and both K_c and K_o are relatively constant among C_3 species and between growth conditions (Berry and Björkman 1980; Yeoh et al. 1981). Given the potential for variation in $V_{c_{\max}}$, P_{m1} and R_d , and the critical role they play in driving the model, we concentrated on these parameters.

Estimating R_d , "day" respiration

The nature of CO_2 -release in the light by processes other than photorespiration remains controversial (Graham 1980). Although we assume in this analysis that mitochondrial respiration continues unabated in the light, recent evidence (Sharpe et al. 1984; Brooks and Farquhar 1985) suggests that it is at least partially inhibited; incorporating such an effect into this analysis would affect the results only slightly. A single measured response of dark mitochondrial respiration in *Arbutus* as a function of leaf temperature is shown in Fig. 1. The curve was fit to Eq. (8) resulting in the parameter values shown in the figure.

Estimating P_m and P_{m1}

The parameter P_m , the CO_2 -saturated rate of CO_2 assimilation at a given irradiance and leaf temperature, is an estimate of the maximum potential rate of RuBP regeneration, and may be described by Eqs. (6) and (7). Ideally, in order to obtain values for the several parameters found in these equations, one should have response curves of CO_2 -saturated net assimilation (P_m) as a function of incident PAR at a wide range of leaf temperatures, including data above the temperature optimum. Lacking such a complete data set for *Arbutus*, we proceeded as follows.

Figure 2 shows the temperature response of P_{m1} , the light- and CO_2 -saturated rate of net photosynthesis (10 mbar $p(\text{O}_2)$) measured on *Quercus suber* (circles), a co-occurring evergreen sclerophyll, with a similar temperature response to *Arbutus* (triangles), but greater photosynthetic capacity on a leaf area basis. The solid line fit to *Quercus* data is the result of a non-linear least squares fit to Eq. (7), resulting in the parameter values shown. The parameters

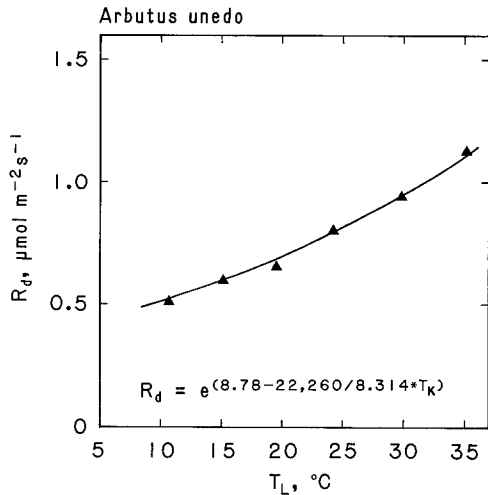


Fig. 1. Measured rates of dark respiration in *Arbutus unedo* as a function of leaf temperature on a single shoot. Solid line is a best least-squares fit to Eq. (8) using values shown

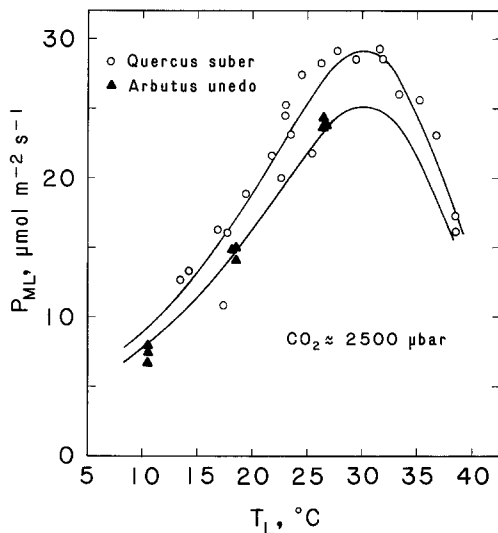


Fig. 2. Measured rates of CO₂- and light-saturated net assimilation (P_{mL}) as a function of leaf temperature for *Quercus suber* (circles) and *Arbutus unedo* (triangles). Top solid line is a fit to *Quercus* data based on Eq. (7); lower line is *Quercus* fit adjusted to fit *Arbutus* data

ΔH_a , ΔS and ΔH_d determine the shape of the response, while the constant, c , adjusts the curve up or down to accommodate differences in photosynthetic capacity. Based on unpublished data indicating that *Arbutus* has temperature characteristics similar to those of *Q. suber*, we adjusted the value of the constant, c , until a suitable fit to the partial curve of *Arbutus* was obtained, as shown. Clearly, applying the *Quercus* fit above 27° C, the highest temperature at which P_{mL} was measured in *Arbutus*, cannot be justified. In fact, however, leaf temperatures experienced in this study exceeded 27° C only rarely and never by more than 2° C.

The parameter P_m is next described using Eq. (6), where P_{mL} is obtained from Eq. (7). The parameter α may be obtained from the initial slope of the net assimilation vs. incident PAR response obtained under CO₂-saturating conditions. We used the value of 0.06 mol CO₂/mol (photons) measured for *Arbutus* leaves in May/June by Beyschlag (1984). A summary of parameter values used to describe P_m and R_d is given in Table 1.

Table 1. Values of model parameters used to calculate "day" respiration (R_d), CO₂-saturated rate of assimilation (P_m) and CO₂- and light-saturated assimilation (P_{mL}) for *Arbutus unedo*

Parameter	Value and units	Used in Equation
α	0.06 mol CO ₂ /mol photons	(6)
c	17.8	(7)
ΔH_a	50,380 J mol ⁻¹	(7)
ΔH_d	197,550 J mol ⁻¹	(7)
ΔS	642.9 J K ⁻¹ mol	(7)
d	8.78	(8)
E	22,260 J mol ⁻¹	(8)

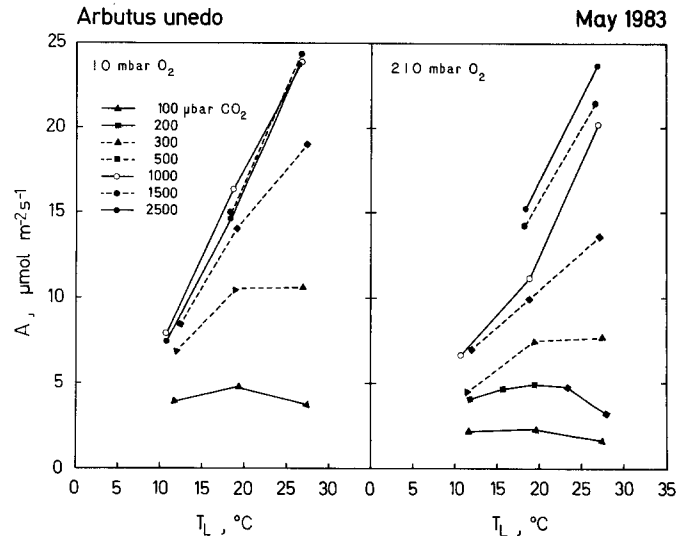


Fig. 3. Net CO₂ assimilation in *Arbutus unedo* as a function of leaf temperature, measured at different $p(\text{CO}_2)$ and either 10 (left panel) or 210 mbar $p(\text{O}_2)$ (right panel). Incident irradiance was saturating or near-saturating ($>1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$)

Estimation of $V_{c_{\max}}$

In order to estimate the temperature dependency of $V_{c_{\max}}$, it was necessary to measure the response of net photosynthesis to internal $p(\text{CO}_2)$ at both 10 and 210 mbar $p(\text{O}_2)$ and several leaf temperatures. In order to obtain such data, incoming $p(\text{CO}_2)$ was maintained constant for a given experiment, $p(\text{O}_2)$ was alternated between 10 and 210 mbar, and steady state rates were obtained at three leaf temperatures, ranging from 11.5 to 27° C. In this way, a family of temperature response curves was obtained at seven $p(\text{CO}_2)$ external (2,500, 1,500, 1,000, 500, 300, 210 and 100 μbar) at both 10 and 210 mbar $p(\text{O}_2)$ (Fig. 3). From these data, response curves of net photosynthesis as a function of internal $p(\text{CO}_2)$, designated P_i , at two $p(\text{O}_2)$ and three leaf temperatures were constructed (Fig. 4).

Adopting the values and temperature dependencies of K_o and K_c obtained in vitro by Badger and Collatz (1977) and utilized by Farquhar et al. (1980), we estimated the temperature dependency of $V_{c_{\max}}$ as follows. The temperature dependencies of K_c , K_o and $V_{c_{\max}}$ may all be described by an Arrhenius function,

$$\text{Parameter} = e^{(f - E_a/RT_K)} \quad (9)$$

where Parameter may represent K_c , K_o or $V_{c_{\max}}$, f is a constant and E_a is activation energy.

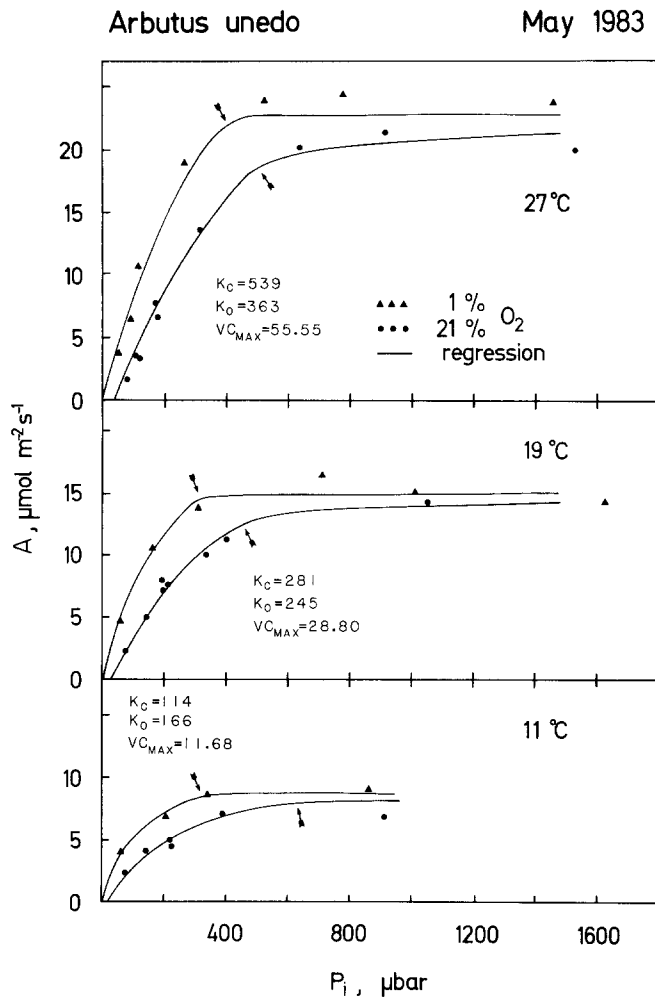


Fig. 4. Net assimilation in *Arbutus unedo* as a function of $p(\text{CO}_2)$ at 10 and 210 mbar $p(\text{O}_2)$ and three leaf temperatures. Curves were assembled from the assimilation responses to leaf temperature at different $p(\text{CO}_2)$ shown in Fig. 3. Lines represent model fits to the data, and arrows denote the transition between the RuBP-limited and RuBP-saturated portions of the response, as predicted by the model

The data of Badger and Collatz (1977) clearly indicate a break at approximately 15°C in the Arrhenius functions describing the temperature dependencies of K_c , $V_{c_{max}}$ and $V_{c_{max}}$, but not K_o . Because our data include points obtained at leaf temperatures above and below 15°C, we incorporated both activation energies for K_c reported by Badger and Collatz, and assumed that the transition from one to

the other occurred at 15°C. Values for the constants and activation energies are given in Table 2.

Using these activation energies and constants and Eq. (9), we calculated the value of K_c and K_o at each of our three measurement leaf temperatures in Fig. 4. Using these values, the initial, linear portions of each of the three pairs of response curves in Fig. 4, was fit to Eq. (4) using least-squares regression techniques which iterated for the best estimate of $V_{c_{max}}$ at each temperature. The calculated values of K_c and K_o and the best-fit value of $V_{c_{max}}$ at each temperature are indicated in Fig. 4.

For the purposes of modeling, it was necessary to describe the temperature dependency of $V_{c_{max}}$ based on these three estimates. Once again assuming an Arrhenius fit with a break at 15°C, we calculated two constants and two activation energies to describe $V_{c_{max}}$, as shown in Table 2. These activation energies for $V_{c_{max}}$ compare quite well with those of Badger and Collatz, and demonstrate the similarity between the temperature dependencies of K_c and $V_{c_{max}}$. A detailed analysis of temperature effects on both RuBP carboxylase-oxygenase and RuBP regeneration may be found in Kirschbaum and Farquhar (1984).

Using the parameter values describing P_{m1} , P_m and R_d (Table 1) and the temperature dependencies of K_c , K_o and $V_{c_{max}}$ (Table 2), Eq. (5) was used to simulate the data in Fig. 4 (solid lines). Note that the line in the initial, carboxylase-limited portion of the curve is not a simulation, but rather a least-squares fit to Eq. (4), used to estimate $V_{c_{max}}$; at higher values of P_i , however, Eq. (5) is used to simulate the data. The break point in the simulated responses, denoted by an arrow and most obvious at the two higher temperature, represents the point of transition between the initial, carboxylase-limited portion of the response and the RuBP-limited portion at higher P_i values.

Simulation of Diurnal Courses of CO_2 Assimilation

Utilizing the values and temperature dependencies for all model parameters, listed in Tables 1 and 2, we employed the model to simulate daily courses of photosynthetic response obtained in the field. Two such diurnal courses are shown in Fig. 5, depicting the measured diurnal pattern of transpiration (E), leaf water potential (ψ), leaf conductance to water vapor (g) and two of the environmental inputs to the model, leaf temperature and incident PAR. P_i is calculated from assimilation and stomatal conductance to CO_2 , equal to $g/1.6$, according to the relationship,

$$P_i = P_a - A_c * g / 1.6 \quad (10)$$

where A_c is calculated CO_2 assimilation. Due to the inherent

Table 2. Values of the constant (f) and the activation energy (E_a) used in Eq. (9) to describe the temperature dependencies of K_c , K_o and $V_{c_{max}}$. A break in the Arrhenius function at 15°C is assumed for K_c and $V_{c_{max}}$ (Badger and Collatz 1977), and $V_{c_{max}}$ is assumed to be 0.21 $V_{c_{max}}$ (Farquhar et al. 1980). Values for K_c and K_o are from Badger and Collatz (1977). Values for $V_{c_{max}}$ are calculated from best least squares estimates of $V_{c_{max}}$ at three leaf temperatures obtained from data fits to Eq. (4)

	Constant (f)		Activation energy (E_a) (J mol^{-1})	
	Above 15°C	Below 15°C	Above 15°C	Below 15°C
K_o	20.32	20.32	36,000	36,000
K_c	30.10	51.08	59,430	109,700
$V_{c_{max}}$	27.97	48.63	59,780	109,300

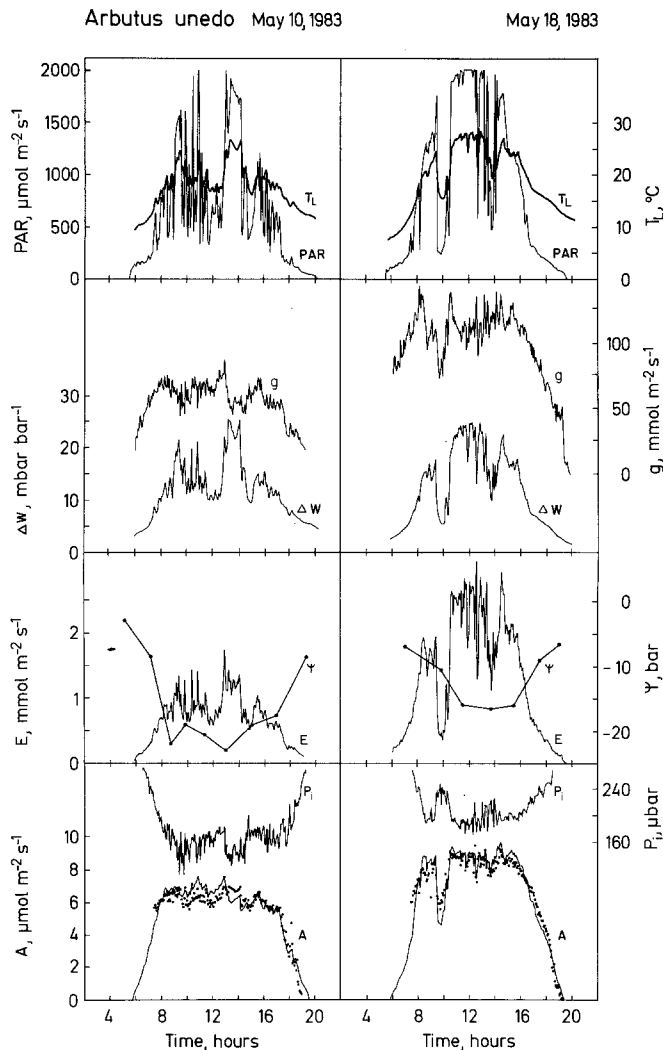


Fig. 5. Daily time courses of incident irradiance (PAR), leaf temperature (T_L), air to leaf water vapor pressure difference (Δw), leaf conductance to water vapor (g), transpiration rate (E), leaf water potential (ψ), net assimilation (A) and calculated internal $p(\text{CO}_2)$ (P_i), measured on a shoot of *Arbutus unedo* under natural conditions for two days in May, 1983. For assimilation, solid line represents measured data and dots represent model predictions

interdependence of P_i and A_c , it was necessary to iterate for the value of P_i to be used in each model prediction. As an initial approximation, $P_i = 100 \mu\text{bar}$ was input to the model and assimilation calculated. Using this value of A_c , P_i was recalculated from Eq. (10) and compared with the initial estimate. This value of P_i was then incremented in steps of $5 \mu\text{bar}$ until the value input to the model and that calculated in Eq. (5) were within $5 \mu\text{bar}$ of each other. Using this value of P_i , and the measured values of PAR and leaf temperature, CO_2 assimilation rate was predicted using Eq. (5). The measured assimilation rates are shown in Fig. 5 as the solid line, and predicted rates appear as closed circles. (Appropriate corrections for effects of transpired water (von Caemmerer and Farquhar 1981) were not incorporated into Eq. (10), leading to slight errors in P_i during the iteration process.)

To further test the ability of the model to predict assimilation under widely varying conditions, we obtained a diurnal response under natural conditions of air temperature and humidity, but with CO_2 entering the cuvette maintained

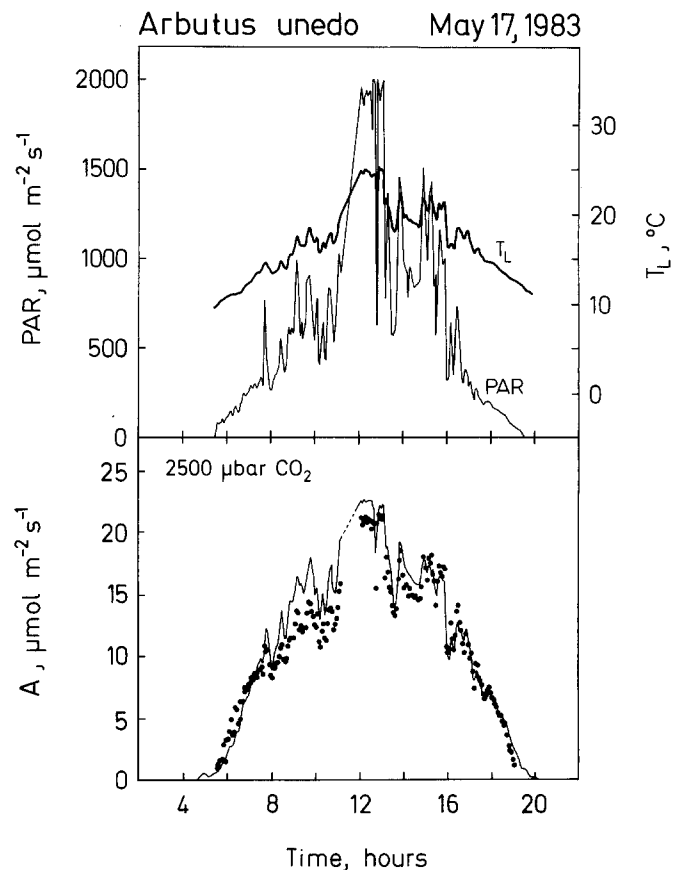


Fig. 6. Daily time course of incident irradiance (PAR), leaf temperature (T_L) and net assimilation for *Arbutus unedo* measured under CO_2 -saturated conditions ($p(\text{CO}_2)$ in cuvette $> 2,500 \mu\text{bar}$). Solid line is measured assimilation; dots are model predictions

at the saturating level of $2,500 \mu\text{bar}$. Measured and predicted assimilation rates and measured values of leaf temperatures and PAR are shown in Fig. 6.

Discussion

Harley et al. (1985) demonstrated that the model of Farquhar et al. (1980) could be successfully applied to simulate whole-leaf photosynthesis in soybean, and that model parameters could be obtained solely through conventional gas-exchange techniques. The primary purpose of the study reported here was to determine whether a protocol could be developed which would allow us to estimate model parameters for *Arbutus* under field conditions, enabling us to simulate diurnal assimilation. We feel the procedure outlined above, employing several simplifying assumptions and concentrating on obtaining the temperature dependencies of $V_{c_{\max}}$, P_{m1} and R_d represents a reasonable compromise between physiological accuracy and field practicality.

In order to apply the model to a given plant requires obtaining (1) dark respiration responses as a function of temperature, (2) response curves relating assimilation to irradiance under conditions of CO_2 saturation at several temperatures, and (3) response curves relating assimilation to P_i over a similar temperature range. Although this study employed A vs. P_i curves at both 10 and 210 mbar $p(\text{O}_2)$, curves at 210 mbar alone might be sufficient, though curves at 10 mbar improve the estimate of $V_{c_{\max}}$. Given recent advances in field gas-exchange methodologies (Field et al.

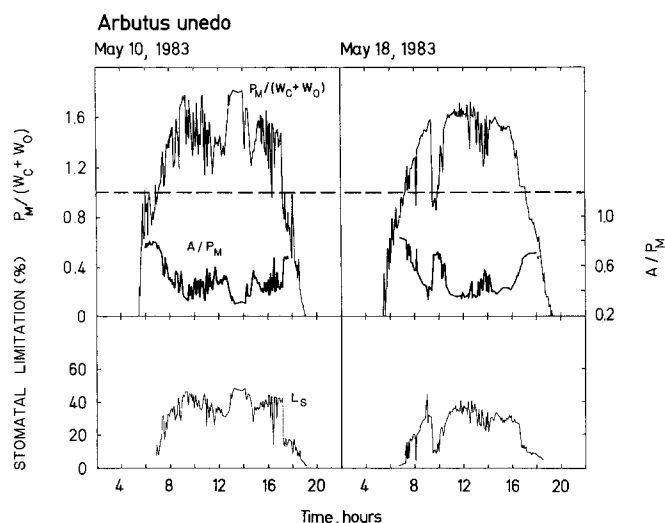


Fig. 7. Daily time course of $P_m/(W_c + W_o)$, A/P_m and stomatal limitation (LS) for the two days depicted in Fig. 5. Dashed line represents a value of 1.0 for $P_m/(W_c + W_o)$

1982; Lange and Tenhunen 1984), obtaining such measurements under field conditions is becoming increasingly practical.

In general, the model simulated quite well diurnal courses of net CO_2 assimilation in *Arbutus*; two typical examples are given in Fig. 5. Note that despite the continuous and occasionally large fluctuations in PAR on both days, the assimilation response was essentially unaffected except for a brief period in the morning on May 18, when PAR dropped below $400 \mu\text{mol m}^{-2} \text{s}^{-1}$, indicating that these shoots become light saturated at quite low irradiances. At the levels of P_i generally prevailing, therefore, RuBP concentration was non-limiting except at irradiances below 400. The model simulates this behavior quite well. Under conditions of saturating $p(\text{CO}_2)$ (Fig. 6), the interactions between RuBP utilization and RuBP regeneration are quite different and assimilation rates 2 to 3 times as high as in ambient air, yet the model performed satisfactorily.

The model developed here allows one to assess those factors responsible for limiting primary productivity. Using the model, it is possible to determine where on the A vs. P_i response curve the shoot is operating throughout the day. The value of P_i is jointly determined by the leaf demand for CO_2 , described by the A vs. P_i response, and the rate of supply of CO_2 , determined via stomatal conductance (Raschke 1979; Farquhar and Sharkey 1982). Farquhar and Sharkey propose a simple method for calculating the stomatal limitation of assimilation (LS), in which the actual assimilation rate (A) at a given time is compared with the rate which would occur were conductance infinite (A_o), i.e., the rate if P_i were equal to ambient $p(\text{CO}_2)$. Thus, expressed as a percentage,

$$LS = (1 - A/A_o) * 100 \quad (11)$$

In order to calculate LS , one needs to know the shape of the response of assimilation to P_i under any given conditions, which is facilitated by an analytical model. The value of LS for each simulated assimilation rate on both May 10 and May 18 was calculated, and these data are plotted in the bottom panel of Fig. 7. On May 10, with conductance values between 50 and $75 \text{ mmol m}^{-2} \text{s}^{-1}$, leading to rela-

tively low values of P_i clustering around $160 \mu\text{bar}$, the value of LS fluctuated around 40%, indicating a 40% reduction in net photosynthesis rate imposed by the stomata. On May 18, with conductances varying between 100 and $125 \text{ mmol m}^{-2} \text{s}^{-1}$ and P_i values clustering around $200 \mu\text{bar}$, the value of LS through the day varied between 25% and 35%, except during the period centered around 10 A.M., at which time reduced irradiance, via its effect on RuBP regeneration, provided the major limitation to photosynthesis, and stomatal limitation fell briefly to values of about 10%. Working with *Arbutus* during a period of increased levels of water stress, Beyschlag (1984) measured A vs. P_i response curves at different times of day. Stomatal limitation was approximately 40% during the morning, but with the onset of midday stomatal closure, stomatal limitation increased to over 80%.

Employing the model, the actual rate of assimilation, A , may be compared with the CO_2 -saturated rate (P_m), where P_m is dependent on irradiance and leaf temperature according to Eqs. (6) and (7). This ratio, A/P_m , was calculated for each data point on May 10 and 18, and these data are shown in the top panel of Fig. 7. The value of A/P_m is below 0.5 for most of both days, rising above 0.6 only when irradiance is low early and late in the day, and during the short period centered around 10 A.M. on May 18, when PAR fell below $400 \mu\text{mol m}^{-2} \text{s}^{-1}$. Tenhunen et al. (1984a) measured the ratio A/P_m in the field in somewhat water-stressed plants of the co-occurring *Quercus suber* under controlled conditions simulating a typical summer day. The ratio showed a similar pattern to that shown in Fig. 7, but with minimum values of below 0.2. In a similar study with *Arbutus*, Beyschlag (1984) measured the ratio of A to P_{m1} , the light- and CO_2 -saturated rate of assimilation, and obtained values ranging from 0.3 to 0.4 during morning hours to values below 0.1 during midday stomatal closure.

Both RuBP carboxylase-oxygenase and those compounds involved in the regeneration of RuBP are "expensive" in terms of nitrogen, a major limiting resource in many ecosystems (Field and Mooney 1986). With respect to efficient allocation of leaf nitrogen to photosynthesis, von Caemmerer and Farquhar (1981) suggest that plants should operate in the region of transition between the RuBP carboxylase-limited and the RuBP regeneration-limited portions of the response, such that the two processes are co-limiting and excess nitrogen is allocated to neither. They demonstrated that, under differing growth conditions with respect to both nitrogen fertilization and irradiance, the operational P_i remained near the transition region in *Phaseolus vulgaris* when measurements were made under conditions similar to those during growth. Harley et al. (1985) reached a similar conclusion using soybean. In a rapidly fluctuating natural environment, however, it is perhaps unrealistic to expect that P_i will remain in this transition region throughout the day. If nitrogen is being allocated efficiently, however, we might expect the operational P_i to remain in the transition region during those times when environmental conditions approximate the "average" conditions experienced in the habitat.

The photosynthesis model allowed us to determine the extent to which *Arbutus unedo* was operating in the transition region. As discussed above, the ratio $P_m/(W_c + W_o)$ compares potential RuBP regeneration capacity (P_m) with potential RuBP utilization in carboxylation and oxygena-

tion ($W_c + W_o$). Thus, when the ratio is equal to one, assimilation is co-limited by the two processes and the operational P_i is at the transition point of the A vs. P_i response. When $P_m/(W_c + W_o)$ is greater than one, RuBP levels are saturating and the shoot is operating on the initial, carboxylase-limited portion of the response, and vice versa. For May 10 and 18, the value of the ratio $P_m/(W_c + W_o)$ was calculated for each simulated assimilation rate, and the results plotted in Fig. 7 (top panel), where the dashed line represents a ratio of one. It is clear that on neither day is the shoot operating near the transition point of the A vs. P_i response curve. Only during sunrise and sunset is irradiance low enough to allow RuBP regeneration to limit assimilation. Despite the fact that PAR on May 10 falls to levels below $500 \mu\text{mol m}^{-2} \text{s}^{-1}$, Fig. 7 indicates that RuBP regeneration remains sufficient to maintain saturating levels of substrate. The single exception occurs at approximately 10 A.M. on May 18, when the ratio drops below the value of one, signifying an RuBP limitation on assimilation, reflected in the concomitant decrease in CO_2 uptake seen in Fig. 5. At this time, levels of PAR have fallen to approximately $250 \mu\text{mol m}^{-2} \text{s}^{-1}$. That *Arbutus* becomes light saturated at relatively low irradiances may be inferred from Fig. 4, where, at leaf temperatures above 19°C , and at typical operational P_i values of 200, the shoots are operating well down on the initial enzyme-limited region of the response, and with a considerable excess of RuBP-regeneration capacity. Thus, even as irradiance is considerably reduced, RuBP regeneration remains sufficient to saturate the carboxylase enzyme.

The above discussion suggests that a fairly gross imbalance exists in these shoots between the concentration of RuBP carboxylase-oxygenase and the capacity to regenerate RuBP, such that they operate most of the time on the RuBP-saturated region of the A vs. P_i response curve. In the relatively high light environment in which these shrubs live, we might expect a greater proportion of their resources, especially nitrogen, to be allocated to RuBP carboxylase-oxygenase, even at the expense of electron transport components or Calvin cycle enzymes used in the regeneration of RuBP. This would increase the slope of the initial carboxylase-limited portion of the A vs. P_i curve and lower the CO_2 -saturated assimilation rate, but would lead to substantial increases in net assimilation at measured rates of stomatal conductance. Similarly, one might conclude that stomatal conductance should also increase, thereby increasing assimilation and driving the operational P_i value closer to the transition point, particularly in the early spring when leaf water potentials are quite high. Because the leaves of *Arbutus* in this experiment were not oriented naturally, however, inferences of this type may not be valid.

The leaves of *Arbutus unedo*, though restrained in a horizontal position during these measurements, naturally incline towards the vertical. This orientation causes the irradiance incident on the leaves during the course of a day to be quite different from that measured in this study, and impossible to predict without having measured angles and azimuth for all leaves in the cuvette. Generally speaking, irradiance incident on the leaves in their natural orientation would have been somewhat greater early and late in the day, and considerably less around solar noon. In terms of the analysis presented above, reduced incident irradiance during the midday would reduce the predicted values of P_m , bringing the value of the ratio $P_m/(W_c + W_o)$ down and

closer to one. Similarly, the values of LS and A/P_m shown in Fig. 7 would be reduced and increased respectively. It is our feeling, however, that under those conditions, this shoot would continue to operate with P_i well down on the initial linear portion of the A vs. P_i response curve for much of the day.

Though it remains unclear why the operational P_i in *Arbutus* is so far down on the carboxylase-limited portion of the A vs. P_i response, this appears to be typical of Portuguese sclerophylls, having also been found in *Arbutus* by Beyschlag (1984) and in *Quercus suber* (Tenhunen et al. 1984a) and *Q. coccifera* (Tenhunen et al. 1984b), although the leaves in each of these studies were also oriented horizontally.

Another reason why the operational P_i may fall on the carboxylase-limited portion of the response relates to efficient CO_2 fixation at low irradiance and during midday depression. During summer drought, photosynthetic production is increasingly confined to early morning and late afternoon hours characterized by relatively low irradiances (Tenhunen et al. 1980c, 1982, 1986). Model simulations suggest that, by having an apparent excess of RuBP regeneration capacity, *Arbutus* is able to utilize more efficiently the lower irradiances which are increasingly important to net productivity as water stress increases. This also suggests that the relative importance of shade leaves to primary production and efficient water use during the summer drought period will be significant. In order for these leaves to operate at the transition point at irradiances of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$, simulations require that the ratio of P_m to $(W_c + W_o)$ at light saturation be 1.8, i.e., an apparent excess of RuBP regeneration capacity.

Although the simulations of diurnal photosynthetic behavior in this study are quite encouraging, it should be mentioned that the model fails to predict accurately CO_2 assimilation during periods of more severe water stress, characterized by midday depression of photosynthesis. It has been demonstrated that midday depression is associated not only with stomatal closure but also with a short-term, reversible decline in both maximum photosynthetic capacity and initial slope of the A vs. P_i response curve (Beyschlag 1984; Resemann and Raschke 1984; Tenhunen et al. 1984a; Tenhunen et al. 1985a; Weber et al. 1985b). Direct water stress effects or effects of leaf to air vapor pressure differences on $V_{c_{\max}}$ or P_{m1} (Lange et al. 1985), perhaps hormonally controlled (Burschka et al. 1985), are not incorporated into the model, with the consequence that the model overestimates net photosynthesis during midday depression.

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