Surfing the Hyperbola Equations of the Steady-State Farquhar–von Caemmerer–Berry C₃ Leaf Photosynthesis Model: What Can a Theoretical Analysis of Their Oblique Asymptotes and Transition Points Tell Us?

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Received: 9 August 2019 / Accepted: 2 December 2019 / Published online: 23 December 2019
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Abstract
The asymptotes and transition points of the net CO₂ assimilation (A/Cᵢ) rate curves of the steady-state Farquhar–von Caemmerer–Berry (FvCB) model for leaf photosynthesis of C₃ plants are examined in a theoretical study, which begins from the exploration of the standard equations of hyperbolae after rotating the coordinate system. The analysis of the A/Cᵢ quadratic equations of the three limitation states of the FvCB model—abbreviated as Aᵢ, Aⱼ and Aₚ—allows us to conclude that their oblique asymptotes have a common slope that depends only on the mesophyll conductance to CO₂ diffusion (gₘ). The limiting values for the transition points between any two states of the three limitation states c, j and p do not depend on gₘ, and the results are therefore valid for rectangular and non-rectangular hyperbola equations of the FvCB model. The analysis of the variation of the slopes of the asymptotes with gₘ casts doubts about the fulfillment of the steady-state conditions, particularly, when the net CO₂ assimilation rate is inhibited at high CO₂ concentrations. The application of the theoretical analysis to extended steady-state FvCB models, where the hyperbola equations of Aᵢ, Aⱼ and Aₚ are modified to accommodate nitrogen assimilation and amino acids export via the photorespiratory pathway, is also discussed.

Keywords  FvCB model · Mesophyll conductance · Leaf photosynthesis · Net CO₂ assimilation rate · Resistance to CO₂ diffusion · Rubisco

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11538-019-00676-z) contains supplementary material, which is available to authorized users.

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1 Introduction

The steady-state Farquhar–von Caemmerer–Berry (FvCB) leaf photosynthesis model is broadly recognised by plant biologists and physiologists as one of the most useful models to assess in vivo the net CO₂ assimilation rate (A) of plant leaves as a function of CO₂ concentration (C) under different environmental cues. The initial FvCB model was first described in the 1980s for C₃ plants (Farquhar et al. 1980), then modified to include the triose phosphate utilisation (Sharkey 1985a, b) and later extended to other works on C₄ plants, antisense transgenic plants, the effect of bicarbonate pumps at the chloroplast envelope and global climate change, among others (Bellasio et al. 2016; Price et al. 2011; von Caemmerer 2000; Wullschleger 1993). Together with the basic rectangular hyperbolic FvCB model (Farquhar et al. 1980; Sharkey 1985a, b), other non-rectangular hyperbolic, exponential and empirical steady-state models have also been described (Duursma 2015; Ethier and Livingston 2004; Goudriaan 1979; von Caemmerer 2000). In the basic FvCB model, the steady-state CO₂ assimilation rate proceeds at the minimum of three limitation rates denoted as Aᵢ, Aⱼ and Aₚ, which depend on the activity of the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the ribulose-1,5-bisphosphate regeneration and the triose phosphate utilisation, hereafter abbreviated as states c, j and p.

In the basic FvCB model, the analysis of the net CO₂ assimilation rate did not consider the (apparent) mesophyll conductance to CO₂ diffusion (gₘ)—hereafter defined as the conductance for CO₂ diffusion from the intercellular space to the site of Rubisco carboxylation assuming that photorespiratory and respiratory CO₂ release occurs in the same compartment as Rubisco carboxylation (von Caemmerer 2013)—and thus, its value was assumed to be infinite. Consequently, the CO₂ concentration in the intercellular (or substomatal) space (Cᵢ) was set equal to the CO₂ concentration at the site of Rubisco carboxylation (Cᵢ) and both rate curves, A/Cᵢ and A/Cᵢ, were not distinguished between each other. The inclusion of a finite value for gₘ into the initial FvCB model transforms Aᵢ and Aᵢ into quadratic equations. This transformation was indeed demonstrated to provide a more accurate estimation of the values for the maximum carboxylation rate (Vₖmax) of Rubisco and the maximum electron transport Jₘₐₓ under steady-state conditions (Ethier and Livingston 2004; Niinemets et al. 2009; von Caemmerer 2000). From a mathematical point of view, the main difference between the equations of the A/Cᵢ and A/Cᵢ rate curves is that the latter are non-rectangular hyperbolae, whose curvature shape in the first quadrant of the Cartesian coordinate system depends on the magnitude of gₘ.

Nowadays, A/Cᵢ, instead of A/Cᵢ, rate curves are extensively used in the estimation of biochemical parameters from leaf photosynthesis, where gₘ is assumed to be finite and purely diffusional and not to depend on the CO₂ concentration inside the leaf. However, the assumption that gₘ remains constant has been challenged in some studies and new extensions have been incorporated into the FvCB model (Flexas et al. 2007; Tholen et al. 2012). For instance, gₘ was proposed to depend on the ratio of mitochondrial CO₂ release to chloroplast CO₂ uptake.
and to decrease particularly at low $C_i$ (Tholen et al. 2012), although other factors, such as the intracellular arrangements of chloroplasts and mitochondria in C$_3$ leaves, were later included in a more generalised model to better explain the dependence of $g_m$ on the above ratio (Yin and Struik 2017). A decrease in the values for $g_m$ was also observed in response to an increase in $C_i$ (Flexas et al. 2007). In this latter study, either modifications in chloroplast shape, which could prevent the chloroplast association with the cell surface, or the involvement of aquaporins, which could facilitate CO$_2$ diffusion across cell membranes by a pH-dependent process, was proposed to regulate the variation of $g_m$. Besides, $g_m$ is tightly co-regulated with the stomatal conductance ($g_s$) (Flexas et al. 2008). $g_s$ varies with both the atmospheric CO$_2$ concentration and the limitation state (Buckley 2017). The value of $g_s$ declines with increased atmospheric CO$_2$ concentration under RuBP regeneration-limited photosynthesis, but, in contrast, it increases with increased atmospheric CO$_2$ concentration under Rubisco-limited photosynthesis (Medlyn et al. 2011).

When the $A/C_i$ rate curves of the FvCB model are analysed under steady-state conditions and the photorespiratory and respiratory CO$_2$ release is also assumed to take place at the site of the Rubisco carboxylation, the quadratic equations for $A_c$, $A_j$ and $A_p$ (see “Appendix 1”, Eqs. A8–A10) can be fitted following different approaches, where $g_m$ is taken as a constant parameter (Duursma 2015; Gu et al. 2010; Sharkey 2016; Su et al. 2009). Some of the nonlinear fitting methods require starting from initial guessed parameters and letting the fit improve with successive iterations, while others constrain the $C_i$ values at which the transition point between c and j occurs. A wealth of data on the transition point between the states c and j indicates that its value is species- and season-dependent, and so it should not be constrained in the fitting method (Duursma 2015; Miao et al. 2009; Zeng et al. 2010). The above fitting methods also take up that the $A/C_i$ rate curves reach asymptotic values for $A$ at supraoptimal CO$_2$ concentration, when there is experimental evidence for the inhibition of the net CO$_2$ assimilation rate by CO$_2$ itself at high concentrations (Woo and Wong 1983). Also, some of these fitting methods make use of approximate estimations for $J_{\text{max}}$ and $T_p$—where $T_p$ stands for the rate of phosphate release in triose phosphate utilisation—when $C_c$ approaches infinity in an $A/C_c$ rate curve (Su et al. 2009) or they reasonably assume that the order of the three limitation states along the $C_i$ axis is the same as along the $C_c$ axis (Gu et al. 2010). Dynamic models of photosynthesis are also suitable to analyse the leaf CO$_2$ assimilation response under fluctuating environmental stimuli such as sunlight irradiance, atmospheric CO$_2$ concentration or stomatal response to light (Bellasio 2019; Morales et al. 2018; Noe and Giersch 2004); however, they add complexity to the analysis or they have not been developed completely to date.

The simplicity of the quadratic equations for $A_c$, $A_j$ and $A_p$ still makes the steady-state FvCB model very useful in fitting approaches to estimate biochemical parameters from leaf photosynthesis (Duursma 2015; Gu et al. 2010; Sharkey 2016; Su et al. 2009). After nearly 40 years of research on the FvCB model, its quadratic equations still hide mathematical features of interest to establish when this model becomes short or when an extended FvCB model would be more suitable for the estimation of the biochemical parameters. On the mathematical analysis of the
The FvCB model we present here, the rotation of the coordinate system has been a key strategy to reach the conclusion that the quadratic equations of the FvCB model cannot explain the inhibition of the net CO$_2$ assimilation rate at very high $C_i$. Also, the mathematical analysis of the limiting conditions for the transition points between $A_c$, $A_j$ and $A_p$ shows that they do not depend on the finite value of $g_m$.

2 Computer Analysis

The computer algebra system Wolfram Mathematica v. 10.3 (Wolfram Research 2015) was used to program scripts to solve analytically the asymptotes and transition points of $A/C_c$ and $A/C_i$ rate curves of the three limitation states c, j and p. Comparative analyses were performed with hyperbolae in standard form after the rotation of the coordinates. The scripts were also run to plot representative $A/C_c$ and $A/C_i$ rate curves. The chosen and finite values for the kinetic constants for Rubisco and other biochemical parameters in the simulations are in the range of those experimentally determined for different C$_3$ plant species (Jahan et al. 2014; von Caemmerer 2000). A list of definitions is given in Table 1 for the sake of clarity.

3 Results and Discussion

3.1 Brief Description of the Asymptotes and Transition Points of the Rectangular Hyperbola Equations of the Basic FvCB Model

According to the basic FvCB model for leaf photosynthesis in C$_3$ plants (Farquhar et al. 1980; Sharkey 1985a, b), the hyperbola equations of the dependence of the net CO$_2$ assimilation rate on the CO$_2$ concentration at the site of the Rubisco carboxylation (i.e. $A/C_c$) are as follows:

$$A = V_c(1 - 0.5\phi) - R_d = \min\{A_c, A_j, A_p\}$$

(1)

with

$$A_c = \frac{V_{cmax}(C_c - \Gamma^*)}{C_c + K_{co}} - R_d,$$

(2)

$$A_j = \frac{J(C_c - \Gamma^*)}{4C_c + 8\Gamma^*} - R_d,$$

(3)

$$A_p = \frac{3T_p(C_c - \Gamma^*)}{C_c - (1 + 3\alpha)\Gamma^*} - R_d,$$

(4)

where $A$ proceeds at a minimum of the three limitation rates $A_c$, $A_j$ and $A_p$. The equations of the three rate curves in the basic FvCB model are branches of rectangular
| Symbol | Definition                                                                 | Units           |
|--------|---------------------------------------------------------------------------|-----------------|
| $A$    | Net CO$_2$ assimilation rate                                              | µmol m$^{-2}$ s$^{-1}$ |
| $A_c$  | Net CO$_2$ assimilation rate assuming Rubisco limitation                  | µmol m$^{-2}$ s$^{-1}$ |
| $A_j$  | Net CO$_2$ assimilation rate assuming ribulose-1,5-bisphosphate regeneration limitation | µmol m$^{-2}$ s$^{-1}$ |
| $A_p$  | Net CO$_2$ assimilation rate assuming triose phosphate use limitation     | µmol m$^{-2}$ s$^{-1}$ |
| $A_{xy}^{*}$ | $A$ at the transition point between any two limitation states (x and y) of the three states c, j and p in the fourth (or third) quadrant of the Cartesian coordinate system. The symbol * stands for chloroplast space (c), intercellular space (i) or atmosphere (a) | µmol m$^{-2}$ s$^{-1}$ |
| $A_{xy}^{*2}$ | As $A_{xy}^{*1}$, except that the transition point takes place in the first quadrant of the Cartesian coordinate system | µmol m$^{-2}$ s$^{-1}$ |
| $C_c$  | Chloroplast CO$_2$ concentration                                          | Pa              |
| $C_i$  | Intercellular CO$_2$ concentration                                         | Pa              |
| $C_a$  | Atmospheric CO$_2$ concentration                                           | Pa              |
| $C_{xy}^{*1}$ | CO$_2$ concentration at the transition point between any two limitation states (x and y) of the three states c, j and p in the fourth (or third) quadrant of the Cartesian coordinate system. * stands for chloroplast (c), intercellular space (i) or atmosphere (a) | Pa |
| $C_{xy}^{*2}$ | As $C_{xy}^{*1}$, except the transition point takes place in the first quadrant of the Cartesian coordinate system | Pa |
| $C_{c2}^{xy}$ | Value of the CO$_2$ concentration when $C_{c2}^{xy}$ approaches $C_{c1}^{xy}$ | Pa |
| $C_{c2}^{xy}$ | Value of the CO$_2$ concentration when $C_{c2}^{xy}$ approaches infinity | Pa |
| $C_{i2}^{xy}$ | Value of the CO$_2$ concentration when $C_{i2}^{xy}$ approaches $C_{i1}^{xy}$ | Pa |
| $C_{i2}^{xy}$ | Value of the CO$_2$ concentration when $C_{i2}^{xy}$ approaches infinity | Pa |
| $J$    | Electron transport rate                                                   | µmol m$^{-2}$ s$^{-1}$ |
| $J_{max}$ | Maximal electron transport rate                                          | µmol m$^{-2}$ s$^{-1}$ |
| $K_c$  | Rubisco Michaelis–Menten constant for carboxylation                      | Pa              |
| $K_o$  | Rubisco Michaelis–Menten constant for oxygenation                         | Pa              |
| $K_{co}$ | Apparent Michaelis–Menten constant                                       | Pa              |
| $O$    | Oxygen concentration                                                     | Pa              |
| $R_d$  | Respiration rate in the light                                             | µmol m$^{-2}$ s$^{-1}$ |
| Symbol | Definition | Units |
|--------|------------|-------|
| $r_m$  | Apparent mesophyll resistance to CO$_2$ diffusion (see the Introduction section). Inverse of $g_m$ | Pa µmol$^{-1}$ m$^2$ s |
| $r_s$  | Stomatal resistance to CO$_2$ diffusion. Inverse of $g_s$ | Pa µmol$^{-1}$ m$^2$ s |
| $T_p$  | Triose phosphate export rate from chloroplasts | µmol m$^{-2}$ s$^{-1}$ |
| $V_{cmax}$ | Maximum carboxylation rate | µmol m$^{-2}$ s$^{-1}$ |
| $y_{asyn}$ | Asymptote with horizontal slope of $A_x$, where $x$ stands for c, j or p | µmol m$^{-2}$ s$^{-1}$Pa$^{-1}$ |
| $y_{asyp}$ | Asymptote with positive slope of $A_x$, where $x$ stands for c, j or p | µmol m$^{-2}$ s$^{-1}$Pa$^{-1}$ |
| $\alpha$ | Fraction of glycerate that does not return to chloroplasts through the photorespiratory cycle | Dimensionless |
| $\beta$ | Angle of rotation of the coordinate system | Dimensionless |
| $\Gamma^*$ | Chloroplast CO$_2$ photocompensation point | Pa |
hyperbolae opening upwards and downwards or left and right, where the coordinate system has been rotated 45° (Appendix 1). The two asymptotes of each of the hyperbolic equations (Eqs. 2–4) are perpendicular to each other with slopes 0 and infinite (Table 2). An elemental analysis of the transition points between the rate equations of the three limitation states gives the following sets of solutions:

For $A_c = A_j$,

$$ C_{c1}^{cj} = \Gamma^* \quad \text{and} \quad A_{c1}^{cj} = -R_d, \quad (5a, 5b) $$

$$ C_{c2}^{cj} = \frac{8V_{cmax} \Gamma^* - JK_{co}}{J - 4V_{cmax}} \quad \text{and} \quad A_{c2}^{cj} = \frac{(J - 4R_d)K_{co} + (J + 8R_d - 12V_{cmax})\Gamma^*}{4K_{co} - 8\Gamma^*} \quad (6a, 6b) $$

for $A_j = A_p$,

$$ C_{c1}^{jp} = \Gamma^* \quad \text{and} \quad A_{c1}^{jp} = -R_d, \quad (7a, 7b) $$

$$ C_{c2}^{jp} = \frac{(J + 24Tp + 3\alpha J)\Gamma^*}{J - 12Tp} \quad \text{and} \quad A_{c2}^{jp} = \frac{12Tp + \alpha J - 4R_d(1 + \alpha)}{4(1 + \alpha)}, \quad (8a, 8b) $$

and for $A_c = A_p$,

$$ C_{c1}^{cp} = \Gamma^* \quad \text{and} \quad A_{c1}^{cp} = -R_d, \quad (9a, 9b) $$

$$ C_{c2}^{cp} = \frac{(1 + \alpha)V_{cmax} \Gamma^* + 3\alpha K_{co}V_{cmax}}{V_{cmax} - 3Tp} \quad \text{and} \quad (10a) $$

$$ A_{c2}^{cp} = \frac{(3(Tp + \alpha V_{cmax}) - (1 + \alpha)R_d)\Gamma^* + (3T_p - R_d)K_{co}}{K_{co} + (1 + \alpha)\Gamma^*} \quad (10b) $$

Together with the transition points ($C_{c1}^{xy}, A_{c1}^{xy}$) between any two limitation states of the three states c, j and p (superscripts x and y) in the first quadrant of the Cartesian coordinate system (subscript 2), there is a common transition point ($C_{c1}^{xy}, A_{c1}^{xy}$) in the fourth quadrant (subscript 1) when $\alpha \neq 0$ (0 ≤ $\alpha$ ≤ 1). Carbon and electron requirements for the assimilation of nitrogen and export of amino acids through the photorespiratory pathway (Busch et al. 2018) are not addressed here, and the standard definition for $\alpha$ in the basic FvCB model remains (see below for further discussion).

### 3.2 Dependence of the Oblique Asymptotes of the Non-rectangular Hyperbola (or Quadratic) Equations of the FvCB Model on $r_m$

The mathematical analysis becomes more challenging if $A/C_1$, instead of $A/C_i$, rate curves are used. When steady-state conditions for CO$_2$ diffusion are achieved, $A_c$, $A_j$ and $A_p$ can be determined after the substitution of $C_i$ for $C_i$ using the equation
Table 2: Summary of the values and equations of the centre, asymptotes and bisecting lines describing the rectangular and non-rectangular hyperbolae of the FvCB model for C3 plants

|                     | Vertical asymptote | Horizontal asymptote | Centre          | Bisecting line                                                                 |
|---------------------|--------------------|----------------------|-----------------|--------------------------------------------------------------------------------|
| **Rectangular hyperbolae** |                    |                      |                 |                                                                                |
| $A_e$               | $C_e = -K_{co}$    | $A_{e\text{asyn}} = V_{cmax} - R_d$ | $( -K_{co}, V_{cmax} - R_d)$ | $A_{e\text{bis}} = -(C_i + K_{co}) + V_{cmax} - R_d$ |
| $A_j$               | $C_j = -2\Gamma^*$ | $A_{j\text{asyn}} = J/4 - R_d$ | $( -2\Gamma^*, J/4 - R_d)$ | $A_{j\text{bis}} = -(C_i + 2\Gamma^*) + J/4 - R_d$ |
| $A_p$               | $C_p = (1 + 3\alpha)\Gamma^*$ | $A_{p\text{asyn}} = 3T_p - R_d$ | $( (1 + 3\alpha)\Gamma^*, 3T_p - R_d)$ | $A_{p\text{bis}} = (C_i - (1 + 3\alpha)\Gamma^*) + 3T_p - R_d$ |

|                     | Vertical asymptote | Horizontal asymptote | Centre          | Bisecting line                                                                 |
|---------------------|--------------------|----------------------|-----------------|--------------------------------------------------------------------------------|
| **Non-rectangular hyperbolae** |                    |                      |                 |                                                                                |
| $A_e$               | $A_{e\text{asyp}} = \frac{C_e + K_{co}}{r_m}$ | $A_{e\text{asyn}} = V_{cmax} - R_d$ | $( (V_{cmax} - R_d)r_m - K_{co}, V_{cmax} - R_d)$ | $A_{e\text{bis}} = -(r_m + \sqrt{1 + r_m^2})(C_i - (V_{cmax} - R_d)r_m + K_{co}) + V_{cmax} - R_d$ |
| $A_j$               | $A_{j\text{asyp}} = \frac{C_j + 2\Gamma^*}{r_m}$ | $A_{j\text{asyn}} = J/4 - R_d$ | $( (J/4 - R_d)r_m - 2\Gamma^*, J/4 - R_d)$ | $A_{j\text{bis}} = -(r_m + \sqrt{1 + r_m^2})(C_i - (J/4 - R_d)r_m + 2\Gamma^*) + J/4 - R_d$ |
| $A_p$               | $A_{p\text{asyp}} = \frac{C_p - (1 + 3\alpha)\Gamma^*}{r_m}$ | $A_{p\text{asyn}} = 3T_p - R_d$ | $( (3T_p - R_d)r_m + (1 + 3\alpha)\Gamma^*, 3T_p - R_d)$ | $A_{p\text{bis}} = -(r_m + \sqrt{1 + r_m^2})(C_i - (3T_p - R_d)r_m + (1 + 3\alpha)\Gamma^*) + 3T_p - R_d$ |
\[ A = \left( C_i - C_c \right) / r_m \] according to Fick’s diffusion law, where the finite and “constant” mesophyll resistance to CO\textsubscript{2} diffusion is \( r_m = 1 / g_m \). Quadratic equations are obtained for \( A_c, A_j \) and \( A_p \) (Eqs. A8–A10). They are now non-rectangular hyperbolae opening upwards and downwards, for the case of \( A_c \) and \( A_j \), and left and right, for the case of \( A_p \), where the coordinate system has now been rotated anticlockwise an angle, here denoted \( \beta \) (Appendix 1). One of the two asymptotes from each non-rectangular hyperbola is parallel to the horizontal axis, but the other is now oblique with a slope exactly equal to the mesophyll conductance to CO\textsubscript{2} diffusion, i.e. \( g_m = 1 / r_m \), a result which is valid for \( A_c \) (von Caemmerer 2000) and also for \( A_j \) and \( A_p \). This conclusion is reached following the analysis of the coefficients of the quadratic equations obtained after the anticlockwise rotation of the coordinate system by \( \beta \). When Eqs. A6 and A7 are compared with Eqs. A8–A10, some key features emerge: firstly, the summation of the coefficients of \( C_i^2 \) is equal to zero and, secondly, the second coefficient of the quadratic equations is, in fact, the summation of the two asymptotes of each hyperbola (Appendix 1). The equations of the two asymptotes for \( A_c, A_j \) and \( A_p \) are therefore summarised as follows:

\[ y_{\text{asyp}}^c = \frac{C_i + K_{co}}{r_m} \quad \text{and} \quad y_{\text{asyn}}^c = V_{\text{cmax}} - R_d, \]  
\[ y_{\text{asyp}}^j = \frac{C_i + 2 \Gamma^*}{r_m} \quad \text{and} \quad y_{\text{asyn}}^j = J/4 - R_d, \]  
\[ y_{\text{asyp}}^p = \frac{C_i - (1 + 3\alpha) \Gamma^*}{r_m} \quad \text{and} \quad y_{\text{asyn}}^p = 3T_p - R_d, \]

where \( y_{\text{asyp}}^c \) and \( y_{\text{asyn}}^c \) stand for the oblique and horizontal asymptotes of \( A_c \), \( A_j \) and \( A_p \), respectively.

It is worth noting that the use of \( \alpha = 0 \) directly in Eq. 4 is an oversimplification of \( A_p \). The oblique asymptote of \( A_p \) is present, even when \( \alpha \) is assumed to be equal to zero (Eq. 13a). The intersection between the two asymptotes of \( A_p \) (i.e. \( y_{\text{asyp}}^p \) and \( y_{\text{asyn}}^p \)), in particular when \( \alpha = 0 \), gives a limiting value below which \( C_i \) is meaningless. In fact, the approximation \( A_p = 3T - R_d \) is not valid in the whole \( C_i \) domain between \( \Gamma^* \leq C_i \leq \infty \). The discontinuity is more obvious when \( \alpha \neq 0 \) because there is a \( C_i \) domain for which no real values for \( A_p \) can be obtained. The suitable \( C_i \) domain for the nonlinear fitting of \( A_p \) in the FvCB model is thus confined to the negative root of its branch opening right (Fig. 1), a result which is also in line with the study by Gu et al. (2010). When \( \alpha \neq 0 \), the values of the negative root of the \( A_p \) branch opening right decrease as \( C_i \) increases.

### 3.3 The Limiting Conditions for the Transition Points in the FvCB Model Do Not Depend on \( r_m \)

The transition points for the negative roots of the quadratic equations for \( A/C_i \) (Eqs. A8–A10) can be solved mathematically and written in a simple form making use of the analytical solutions (Eqs. 5–10) for \( A/C_c \) as follows:
For $A_c = A_j$,

\[ C_{i1}^{cj} = C_{c1}^{cj} + r_m A_{c1}^{cj} \quad \text{and} \quad A_{i1}^{cj} = A_{c1}^{cj}, \]  
\[ C_{i2}^{cj} = C_{c2}^{cj} + r_m A_{c2}^{cj} \quad \text{and} \quad A_{i2}^{cj} = A_{c2}^{cj}, \]  
\[ C_{i1}^{jp} = C_{c1}^{jp} + r_m A_{c1}^{jp} \quad \text{and} \quad A_{i1}^{jp} = A_{c1}^{jp}, \]  
\[ C_{i2}^{jp} = C_{c2}^{jp} + r_m A_{c2}^{jp} \quad \text{and} \quad A_{i2}^{jp} = A_{c2}^{jp}, \]  
\[ C_{i1}^{cp} = C_{c1}^{cp} + r_m A_{c1}^{cp} \quad \text{and} \quad A_{i1}^{cp} = A_{c1}^{cp}, \]  
\[ C_{i2}^{cp} = C_{c2}^{cp} + r_m A_{c2}^{cp} \quad \text{and} \quad A_{i2}^{cp} = A_{c2}^{cp}. \]  

The above solutions could be further extended to include the stomatal resistance to CO₂ diffusion ($r_s = 1/g_s$) in $A_c$, $A_j$ and $A_p$ (see Appendix 2). Figure 2 summarises the changes in the transition points in the first and fourth quadrants of the Cartesian coordinate system when the resistance(s) to CO₂ diffusion is included in the net CO₂ assimilation rate curves. For the sake of clarity, only $A_c$ and $A_j$ are shown.
The analytical values for the assimilation rates \( (A_{xy}^1) \) and \( (A_{xy}^2) \) at the transition points remain constant, while the values for the CO\(_2\) concentration increase in the first quadrant (and decrease in the fourth quadrant) when the resistance(s) to CO\(_2\) diffusion increases. The solution \( (C_{xy}^1, A_{xy}^1) \) for the transition point \( (C_{xy}^1, A_{xy}^1) \) is restricted to the fourth quadrant of the Cartesian coordinate system when the rectangular hyperbolae (Eqs. 2–4) of the basic FvCB model are used. However, it should not be surprising to find out this analytical transition point \( (C_{xy}^1, A_{xy}^1) \) in the third quadrant under conditions for which \( R_d r_m > \Gamma^* \) when dealing with the quadratic equations of the FvCB model.

In the mathematical analysis, it can be observed that the common transition point \( (C_{xy}^1, A_{xy}^1) \) between the three states c, j and p is always present; in contrast, the transition points \( (C_{xy}^2, A_{xy}^2) \) depend on the biochemical parameters and might not be present in the net CO\(_2\) assimilation rate curves. Two limiting conditions can now be investigated in order to analyse all the possible combinations between the transition points between \( A_c, A_j \) and \( A_p \) regardless of the value of \( r_m \). One is that \( C_{xy}^2 \) approaches \( C_{xy}^1 \) (i.e. \( C_{xy}^2 \rightarrow \infty \)) and another is that \( C_{xy}^2 \) approaches infinity (i.e. \( C_{xy}^2 \rightarrow \infty \)). The equation \( C_{xy}^1 = C_{xy}^2 \) has to be solved for the analysis of the first limiting condition, whereas only the values for the
denominator of the first summand (i.e. $C_{i2}^{y}$) of $C_{i2}^{y}$ (Eqs. 15a–19a) have to be inspected to analyse the second limiting condition. In the analysis, the constraint $K_{co} > 2 \Gamma^*$ is imposed based on the values reported for C₃ plants (von Caemmerer 2000); consequently, there are no finite values for the biochemical parameters of the second summand (i.e. $r_m A_{i2}^{y}$) of $C_{i2}^{y}$ (Eqs. 15a–19a) that can make this summand approach infinity.

The ratios between the biochemical parameters to reach the above limiting conditions for the rectangular equations of an $A/C_c$ rate curve (i.e. $C_{c2→1}$ and $C_{c2→∞}$) can be derived straightforward from Eqs. 5a–10a. The results are as follows:

For $C_{c2→1}$ and $C_{c2→∞}$, respectively,

$$V_{cmax} = \frac{J(K_{co} + \Gamma^*)}{12 \Gamma^*} \quad \text{and} \quad V_{cmax} = \frac{J}{4}$$  \hspace{1cm} (20a, 20b)

for $C_{c2→1}$ and $C_{c2→∞}$, respectively,

$$J = -\frac{12T_p}{\alpha} \quad \text{and} \quad J = 12T_p$$  \hspace{1cm} (21a, 21b)

and for $C_{c2→1}$ and $C_{c2→∞}$, respectively,

$$V_{cmax} = -\frac{T_p(K_{co} + \Gamma^*)}{\alpha \Gamma^*} \quad \text{and} \quad V_{cmax} = 3T_p$$  \hspace{1cm} (22a, 22b)

Among the above ratios (Eqs. 20a–22b), the ratios between the biochemical parameters for $C_{c2→1}$ and $C_{c2→∞}$ are of non-biochemical significance. They imply that there should be conditions for which one could expect triose phosphate import to chloroplasts (i.e. $T_p < 0$). In fact, if these transition points are analysed, particularly, in a non-rectangular $A/C_i$ rate curve, one can observe that both transitions, ($C_{i2}^{y}, A_{i2}^{y}$) and ($C_{i1}^{y}, A_{i1}^{y}$), occur with the negative root of the hyperbolic branch opening left of $A_p$, for which the $C_i$ domain is not applicable as indicated above. (Further details are given in Fig. S1 of Online Resource.)

When the rest of the ratios between the biochemical parameters are now investigated in the non-rectangular hyperbola equations of the FvCB model—where $r_m$ is finite, $0 < r_m < \infty$—two solutions are in fact found for any equation like $C_{i2}^{y} = C_{i2}^{y}$, of which only one also fulfils the condition $A_{i2}^{y} = A_{i1}^{y}$ (data not shown). The analysis indeed shows that the correct ratios between the biochemical parameters for $C_{i2→1}$ and $C_{i2→∞}$ are the same as those found for $C_{c2→1}$ and $C_{c2→∞}$ (Eqs. 20a–22b). This means that the ratios between the biochemical parameters in the two limiting conditions do not depend on the value of $r_m$. The limiting conditions for the transition points can therefore be reduced as follows:

$$V_{cmax} = \frac{J(K_{co} + \Gamma^*)}{12 \Gamma^*}, \quad \text{and} \quad J = 4V_{cmax}$$  \hspace{1cm} (23, 24)
The graphic representation of $A_c$, $A_j$ and $A_p$ for $A/C_i$ rate curves shows, firstly, that there are no experimental ratios for the biochemical parameter for which $A_p$ (with $T_p > 0$) can be the only limitation state along the domain $r_m R_d < C_i < \infty$ and, secondly, there are ratios between the biochemical parameters for which $A_c$ or $A_j$ can be the only limitation state along the domain $r_m R_d < C_i < \infty$ (Fig. 3a, b). Additional ratios between the biochemical parameters can be found for which there are one or two transition points in the first quadrant of Cartesian coordinate system.

$$J = 12T_p$$  \hspace{1cm} (25)

![Diagram showing ratios between the limiting values of biochemical parameters](image)

**Fig. 3** Ratios between the limiting values of the biochemical parameters of a representative $A/C_i$ rate curve, where the summation of resistances to CO$_2$ diffusion is included (i.e. $0 < r_m < \infty$), for which there are no transition points between the three states c, j and p (a, b) and two transition points (c) or there is only one transition point (d–f) in the first quadrant of the Cartesian coordinate system ($C_{i2}^x$, $A_{i2}^y$). The symbols x and y stand for any of the three limitation states c, j and p. The simulation was performed using the following values for the biochemical parameters: $V_{\text{max}}$, 36–100 $\mu$mol m$^{-2}$ s$^{-1}$; $J$, 24–144 $\mu$mol m$^{-2}$ s$^{-1}$; $T_p$, 8–12 $\mu$mol m$^{-2}$ s$^{-1}$; $R_d$, 2 $\mu$mol m$^{-2}$ s$^{-1}$; $r_m$, 0.4 Pa $\mu$mol$^{-1}$ m$^2$ s; $K_{co}$, 62.1 Pa; $\Gamma^*$, 3.74 Pa
(Fig. 3c–f). The latter ratios are equivalent to those discussed before for $A/C_c$ rate curves (Gu et al. 2010). Regardless of the number of transition points (0, 1 or 2) that the ratios of the biochemical parameters can yield between the three limitation states in the first quadrant of the Cartesian coordinate system, the transition points in the fourth (or third) quadrant are always present.

3.4 Analysis of the Inhibition of the Net CO$_2$ Assimilation Rate at High CO$_2$ Concentrations

If the steady-state FvCB model is strictly followed, one can state, first, that the slopes of the oblique asymptotes of the non-rectangular hyperbolae only depend on $g_m = 1/r_m$, while the slopes of the horizontal asymptotes of $A_c$, $A_j$ and $A_p$ remain unchanged regardless of the value for $g_m$ (Eqs. 11a–13b) and, second, the slopes of the bisecting lines (Table 2) of $A_c$, $A_j$ and $A_p$ correspond with the angle (or the perpendicular angle) of the rotation of the coordinate system that makes the summation of the coefficients of $C_i^2$ equal to zero (Eqs. A11 and A12). This means that there are no mathematical solutions for the quadratic equations of $A_c$, $A_j$ and $A_p$ (Eqs. A8–A10) in the FvCB model for which the slopes of the horizontal asymptotes can be modified to reach negative values. The fraction of glycerate ($\alpha \neq 0$) that does not return to chloroplasts through the photorespiratory cycle (Harley and Sharkey 1991) makes $A_p$ decrease as $C_i$ increases, but the slope of the horizontal asymptote of $A_p$ remains unchanged, no matter what value $\alpha$ ($0 \leq \alpha \leq 1$) has. This indicates that $A_p$ must finally reach a constant value as $C_i$ increases. This conclusion also applies to the extended FvCB model described in the study by Busch et al. (2018), where the parameter $\alpha$ of the basic FvCB model is replaced with two new parameters $\alpha_G$ and $\alpha_S$ that stand for the proportion of glycolate carbon taken out of the photorespiratory pathway as glycine, and the proportion taken out as serine, respectively. Although $\alpha_G$ and $\alpha_S$ might not be constant and depend on the photorespiratory pathway and the reduction of supplied nitrate (Busch et al. 2018), the new equations for the three limitation states remain, from a mathematical point of view, as rectangular hyperbolae with horizontal asymptotes equivalent to those summarised in Table 2. Likewise, the extension of the FvCB model using $r_m$ as a flux-weighted quantity that depends on mitochondrial respiration and photorespiration effects does not explain the inhibition of $A/C_i$ at high $C_i$ either (Tholen et al. 2012).

Despite what has been said above, there are lines of experimental evidence that indicate that negative slopes can be indeed observed in $A/C_i$ rate curves. Woo and Wong (1983) showed that supraoptimal CO$_2$ concentrations inhibited the net CO$_2$ assimilation in cotton plants, and they proposed that an acidification mechanism mediated by CO$_2$ could affect both the thylakoid electron transport and the activity of key enzymes of the Calvin–Benson–Bassham cycle (Kaiser and Heber 1983; Ögren and Evans 1993). At this point, one could speculate on some mathematical explanations for negative slopes in experimental $A/C_i$ rate curves. In the first place, one could wonder whether other rotation of the coordinates—different from the one that yields Eqs. A28 and A29—would be possible under steady-state conditions, which rendered negative asymptotes instead of horizontal asymptotes. If this were
possible, the summation of the coefficients of $C_i^2$ (Eqs. A11 and A12) would not be zero and so the chosen fitting method should start from extended quadratic equations as Eqs. A6 and A7, where at least four parameters defining the hyperbola equation and an angle of rotation had to be determined. In this case, the angle of rotation should depend on $g_m$ together with other biochemical parameters. Alternatively, one could wonder whether the steady-state conditions do not hold along the whole $C_i$ domain, particularly at supraoptimal $CO_2$ concentrations. If this were the case, one can assert that the equation $A = (C_i - C_c)/r_m$ is not always valid. So, $A$ decreases at supraoptimal $CO_2$ concentrations because either $r_m$ is not only diffusional and so it increases as $C_i$ increases (Flexas et al. 2007) or the photosynthetic activity is indeed inhibited by $CO_2$ acidification (Kaiser and Heber 1983; Ögren and Evans 1993; Woo and Wong 1983). Reliable nonlinear fittings of $A_c$ and $A_j$ of the FvCB model can thus be possibly obtained under steady-state conditions using standard approaches (Duursma 2015; Gu et al. 2010; Sharkey 2016; Su et al. 2009); however, the use supraoptimal $CO_2$ concentrations to fit $A_p$ might cast doubts on the fitted biochemical parameters if evidence for negative slopes in the experimental $A/C_i$ rate curves is observed. Based on the variation of $g_m$ with $C_i$ (Flexas et al. 2007), other nonlinear fitting approaches proposed the combination of gas exchange methods with chlorophyll fluorescence-based methods to estimate $g_m$ by using only data within the $j$ state (Yin and Struik 2009).

### 4 Conclusions

The analysis of the steady-state FvCB model for $C_3$ plants starting from the standard equations of hyperbolae after rotating the coordinate system has disclosed some features hidden in the quadratic equations of $A_c$, $A_j$ and $A_p$ of the $A/C_i$ rate curves. In particular, academic interest has been the angle of the rotation of the coordinate system from which it has been established that the oblique asymptotes of the three limitation rate curves share a common slope whose value depends only on $g_m$. $A_p$ always has an oblique asymptote regardless of the value of $\alpha$. The limiting conditions for the transition points in the FvCB model do not depend on $g_m$. The hyperbola equations of $A_c$, $A_j$ and $A_p$ in the FvCB model or in some of the extended steady-state FvCB models here discussed can only provide horizontal asymptotes when the $CO_2$ concentration approaches infinity when, in contrast, there is experimental evidence for negative slopes in $A/C_i$ rate curves at high $CO_2$ concentrations. This leads us to the conclusion that extended quadratic equations containing a $C_i^2$ term might be required for the analysis of $A_c$, $A_j$ and $A_p$ or, in contrast, that steady-state conditions do not hold, particularly, with increased $CO_2$ concentrations. Dynamic modelling taking into account the decrease in the values for $g_m$ or the activity inhibition of key enzymes of the Calvin–Benson–Bassham cycle by $CO_2$ acidification could alternatively provide suitable models for the estimation of the biochemical parameters from leaf photosynthesis.

**Acknowledgements** This study was funded by the Spanish National Research, Development and Innovation Plan of the Ministry of Economy and Competitiveness (Grant Numbers AGL2013-41363-R,
AGL2016-79589-R). ELM-B received a predoctoral contract from the Junta de Castilla y León (E-37-2017-0066125).

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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Appendix 1

The equation of a hyperbola in standard form is as follows:

$$\frac{(x - h)^2}{a^2} - \frac{(y - k)^2}{b^2} = 1,$$  \hspace{1cm} \text{when it opens left and right or} \hspace{1cm} (A1)

$$\frac{(y - k)^2}{b^2} - \frac{(x - h)^2}{a^2} = 1,$$  \hspace{1cm} \text{when it opens upwards and downwards} \hspace{1cm} (A2)

For each hyperbola, there are two asymptotes with slopes of $\pm b/a$ forming an angle $\theta$ whose value is:

$$\theta = \arctan \left( \frac{2ab}{a^2 - b^2} \right)$$  \hspace{1cm} (A3)

The anticlockwise rotation of the coordinates by an angle $\beta$, where

$$\dot{x} = x \cos \beta + y \sin \beta, \quad \text{and}$$  \hspace{1cm} (A4)

$$\dot{y} = -x \sin \beta + y \cos \beta,$$  \hspace{1cm} (A5)

yields:

$$y^2_{LR} + y^2_{LR} \frac{2a^2 \cos \beta \sin \beta + 2b^2 \cos \beta \sin \beta \cdot x + 2a^2 k \cos \beta - 2b^2 h \sin \beta}{b^2 \sin^2 \beta - a^2 \cos^2 \beta}$$

$$+ \frac{(b^2 \cos \beta - a^2 \sin^2 \beta) \cdot x^2 - (2b^2 h \cos \beta + 2a^2 k \sin \beta \cdot x + b^2 h^2 - a^2 k^2 - a^2 b^2}{b^2 \sin^2 \beta - a^2 \cos^2 \beta} = 0$$  \hspace{1cm} (A6)

and
\[
\begin{align*}
    y^2_{\text{UD}} + y_{\text{UD}} &= \frac{(2a^2 \cos \beta \sin \beta + 2b^2 \cos \beta \sin \beta)x + 2a^2 k \cos \beta - 2b^2 h \sin \beta}{b^2 \sin^2 \beta - a^2 \cos^2 \beta} + \frac{(b^2 \cos^2 \beta - a^2 \sin^2 \beta)x^2 - (2b^2 h \cos \beta + 2a^2 k \sin \beta)x + b^2 h^2 - a^2 k^2 + a^2 b^2}{b^2 \sin^2 \beta - a^2 \cos^2 \beta} = 0,
\end{align*}
\] 

where LR and UD stand for hyperbolae opening left and right, and upwards and downwards, respectively. The only difference between the left-hand sides of Eqs. A6 and A7 is the sign of the term \(a^2b^2\) in the numerator of the last coefficient. The above quadratic equations for non-rectangular hyperbolae opening left and right or upwards and downwards can now be compared, coefficient by coefficient, with each of the quadratic equations of the \(A/C_i\) rate curves of the three states c, j and p of the steady-state FvCB model. After the substitution of \(C_c\) with \(C_i - r_m A\) in Eqs. 2–4, the new \(A_c, A_j\) and \(A_p\) are as follows:

\[
\begin{align*}
    A^2_c - A_c \left( \frac{C_i + K_{\text{co}}}{r_m} + V_{\text{cmax}} - R_d \right) + \frac{(V_{\text{cmax}} - R_d) C_i}{r_m} - \frac{V_{\text{cmax}} \Gamma^* + R_d K_{\text{co}}}{r_m} &= 0, \\
    A^2_j - A_j \left( \frac{C_i + 2 \Gamma^*}{r_m} + J/4 - R_d \right) + \frac{(J/4 - R_d) C_i}{r_m} - \frac{(J/4 + 2R_d) \Gamma^*}{r_m} &= 0, \quad \text{and} \\
    A^2_p - A_p \left( \frac{C_i - (1 + 3\alpha) \Gamma^*}{r_m} + 3T_p - R_d \right) + \frac{(3T_p - R_d) C_i}{r_m} - \frac{(3T_p - R_d) \Gamma^*}{r_m} &= 0
\end{align*}
\]

In the first instance, it is observed that the summation of the coefficients of \(C_i^2\) in each of the three rate curves is zero (Eqs. A8–A10). Therefore, the rotation of the coordinates has to fulfil the condition:

\[
b^2 \cos^2 \beta - a^2 \sin^2 \beta = 0, \quad \text{and so}
\]

\[
\beta = \pm \arctan \frac{b}{a} + n\pi, \quad \text{where} \quad n = 0, 1, 2, \ldots
\]

This implies that one of the two asymptotes of \(A_c, A_j\) and \(A_p\) is now parallel to the horizontal axis of the Cartesian coordinate system (i.e. the slope \(m_{\text{asy1}} = 0\). Because the rotation does not change the angle between the two asymptotes of the hyperbolae (Eq. A3), \(\theta\) remains constant and so the slope of the second asymptote \(m_{\text{asy2}}\) has to be as follows:

\[
m_{\text{asy2}} = \pm 2ab / (a^2 - b^2)
\]

The rotation of the coordinates was chosen to be anticlockwise and so the positive value only applies here. The slopes \(m_{\text{asy1}}\) and \(m_{\text{asy2}}\) will be renamed \(m_{\text{asyn}}\) and \(m_{\text{asy2}}\), respectively, after the rotation of the coordinates (see below). Figure 4 summarises the
main changes in the graphic representation of the two types of hyperbolae and their asymptotes after the rotation of the coordinates.

In the second instance, $A_c$, $A_j$ and $A_p$ share a common term within the second coefficient of the quadratic equations of $A_c$, $A_j$ and $A_p$ of the (non)-rectangular hyperbolic FvCB model for C_3 plants. $A_c$ and $A_j$ are rotated hyperbolae opening upwards and downwards, while $A_p$ opens left and right (see text for further details).

\[
2a^2 \cos \beta \sin \beta + 2b^2 \cos \beta \sin \beta = -\frac{1}{r_m} \tag{A14}
\]

The anticlockwise rotation of the coordinates by $\beta = \arctan b/a + n\pi$, where $n = 0$, yields:

\[
\frac{2ab}{a^2 - b^2} = \frac{1}{r_m} \tag{A15}
\]
This solution indicates that the oblique asymptotes of three $A_c, A_j$ and $A_p$ rate curves have in common their slope, which results equal to the mesophyll conductance to CO$_2$ diffusion ($g_m = 1/r_m$).

In the third instance, the second coefficient of each quadratic rate curve is in fact the negative value of the sum of its two asymptotes. If Eqs. A4 and A5 are applied to both the negative and positive asymptotes of the standard forms of the hyperbolae (Eqs. A1 and A2), the following equations are obtained:

$$y_{\text{asyn}} = \frac{ak + bh}{a \cos \beta + b \sin \beta} + \frac{a \sin \beta - b \cos \beta}{a \cos \beta + b \sin \beta} x = n_{\text{asyn}} + m_{\text{asyn}} x, \quad \text{and} \quad (A16)$$

$$y_{\text{asyp}} = \frac{ak - bh}{a \cos \beta - b \sin \beta} + \frac{a \sin \beta + b \cos \beta}{a \cos \beta - b \sin \beta} x = n_{\text{asyp}} + m_{\text{asyp}} x, \quad (A17)$$

where $y_{\text{asyn}}$ and $y_{\text{asyp}}$ stand for the new asymptotes after the rotation of the coordinates by $\beta$ (Fig. 4). The sum of the asymptotes is equal to the negative value of the summation of the coefficients of $y_{\text{LR}}$ and $y_{\text{UD}}$ in Eqs. A6 and A7:

$$y_{\text{asyn}} + y_{\text{asyp}} = -\frac{(2a^2 \cos \beta \sin \beta + 2b^2 \cos \beta \sin \beta) x + 2a^2 k \cos \beta - 2b^2 h \sin \beta}{b^2 \sin^2 \beta - a^2 \cos^2 \beta}$$

(A18)

If, particularly, $\beta = \arctan b/a$, then the slopes have the following values in Eqs. A16 and A17: $m_{\text{asyn}} = 0$ and $m_{\text{asyp}} = 2ab/(a^2 - b^2)$. So now we can rewrite the second coefficients of the $A_c, A_j$ and $A_p$ rate curves as follows:

$$y_{\text{asyn}}^c + y_{\text{asyn}}^p = \frac{C_i + K_{\text{co}}}{r_m} + V_{\text{cmax}} - R_d \quad (A19)$$

$$y_{\text{asyn}}^j + y_{\text{asyn}}^p = \frac{C_i + 2I^*}{r_m} + J/4 - R_d \quad (A20)$$

$$y_{\text{asyn}}^p + y_{\text{asyn}}^p = \frac{C_i - (1 + 3\alpha)I^*}{r_m} + 3T_p - R_d, \quad (A21)$$

where the superscript indicates the name of the states c, j or p.

In order to know individually the equations for each of the two asymptotes of $A_c, A_j$ and $A_p$, one can derive the values of the negative asymptotes ($y_{\text{asyn}}^*) from the term that multiplies $x$ in the third coefficient of the quadratic equations (Eqs. A6 and A7). This term is, in fact, the negative value of the product between Eqs. A14 and A16, when $m_{\text{asyn}} = 0$. Therefore, Eqs. A19–A21 can now be split as follows:

$$y_{\text{asyn}}^c = \frac{C_i + K_{\text{co}}}{r_m} \quad \text{and} \quad (A22)$$
The intersection between the oblique and horizontal asymptotes can be used to know the centre of the hyperbolae. The slopes of the bisecting lines are as follows:

\[ y_{\text{asyn}}^c = V_{\text{cmax}} - R_d, \quad \text{(A23)} \]

\[ y_{\text{asyn}}^j = \frac{C_i + 2\Gamma^*}{r_m} \text{ and} \quad \text{(A24)} \]

\[ y_{\text{asyn}}^j = \frac{J}{4} - R_d, \quad \text{and} \quad \text{(A25)} \]

\[ y_{\text{asyn}}^p = \frac{C_i - (1 + 3\alpha)\Gamma^*}{r_m} \text{ and} \quad \text{(A26)} \]

\[ y_{\text{asyn}}^p = 3T_p - R_d. \quad \text{(A27)} \]

The intersection between the oblique and horizontal asymptotes can be used to know the centre of the hyperbolae. The slopes of the bisecting lines are as follows:

\[ m_{\text{bis}}^x = -r_m - \sqrt{1 + r_m^2}, \quad \text{where} \quad x \text{ stands for } c \text{ or } j, \quad \text{and} \quad \text{(A28)} \]

\[ m_{\text{bis}}^p = -r_m + \sqrt{1 + r_m^2} \quad \text{(A29)} \]

The vertices of each hyperbola can also be derived from the intersections between the equation of the bisecting line—passing through the vertices and the centre of the hyperbola—and the corresponding quadratic equation (Eqs. A8–A10). Table 2 includes a summary of the equations and values that describe the rectangular and non-rectangular hyperbolic equations of the FvCB model.

**Appendix 2**

Under steady-state conditions, the net CO₂ assimilation rate can also be obtained as

\[ A = (C_a - C_i)/r_s, \quad \text{where} \quad r_s = 1/g_s, \quad \text{if particularly the transpiration rate} \ (E) \ \text{is considered negligible and consequently} \ g_s \pm E/2 \approx g_s \ \text{(Farquhar and Sharkey 1982).} \]

The transition points for the new equations in \( A/C_a \) rate curves, when both \( r_m \) and \( r_s \) are grouped together in the analysis, are now as follows:

For \( A_c = A_j \)

\[ C_{a1}^{cj} = C_{c1}^{cj} + (r_s + r_m)A_{c1}^{cj} \quad \text{and} \quad A_{a1}^{cj} = A_{c1}^{cj} \quad \text{(A30a, A30b)} \]

\[ C_{a2}^{cj} = C_{c2}^{cj} + (r_s + r_m)A_{c2}^{cj} \quad \text{and} \quad A_{a2}^{cj} = A_{c2}^{cj} \quad \text{(A31a, A31b)} \]

for \( A_j = A_p \).
\[ C_{a1}^{jp} = C_{c1}^{jp} + (r_s + r_m)A_{c1}^{jp} \quad \text{and} \quad A_{a1}^{jp} = A_{c1}^{jp} \quad (A32a, A32b) \]
\[ C_{a2}^{jp} = C_{c2}^{jp} + (r_s + r_m)A_{c2}^{jp} \quad \text{and} \quad A_{a2}^{jp} = A_{c2}^{jp} \quad (A33a, A33b) \]

and for \( A_c = A_p \),

\[ C_{a1}^{cp} = C_{c1}^{cp} + (r_s + r_m)A_{c1}^{cp} \quad \text{and} \quad A_{a1}^{cp} = A_{c1}^{cp} \quad (A34a, A34b) \]
\[ C_{a2}^{cp} = C_{c2}^{cp} + (r_s + r_m)A_{c2}^{cp} \quad \text{and} \quad A_{a2}^{cp} = A_{c2}^{cp} \quad (A35a, A35b) \]

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