2 **Nonlinear dynamics of the CAM circadian rhythm in response to**
3 **environmental forcing**

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Abstract

Crassulacean acid metabolism (CAM) photosynthesis functions as an endogenous circadian rhythm coupled to external environmental forcings of energy and water availability. This paper explores the nonlinear dynamics of a new CAM photosynthesis model [2] and investigates the responses of CAM plant carbon assimilation to different combinations of environmental conditions. The CAM model [2] consists of a Calvin cycle typical of C3 plants coupled to an oscillator of the type employed in the Van der Pol and FitzHugh-Nagumo systems. This coupled system is a function of environmental variables including leaf temperature, leaf moisture potential, and irradiance. Here, we explore the qualitative response of the system and the expected carbon assimilation under constant and periodically forced environmental conditions. The model results show how the diurnal evolution of these variables entrains the CAM cycle with prevailing environmental conditions. While constant environmental conditions generate either steady-state or periodically oscillating responses in malic acid uptake and release, forcing the CAM system with periodic daily fluctuations in light exposure and leaf temperature results in quasi-periodicity and possible chaos for certain ranges of these variables. This analysis is a first step in quantifying changes in CAM plant productivity with variables such as the mean temperature, daily temperature range, irradiance, and leaf moisture potential. Results may also be used to inform model parametrization based on the observed fluctuating regime.

KEY WORDS: crassulacean acid metabolism (CAM) photosynthesis, circadian rhythm oscillator, chaos, quasi-periodicity, bifurcation

1. Introduction

Because of their high water use efficiency and often succulent nature, crassulacean acid metabolism (CAM) plants thrive in environments where water is limiting, achieving a greater net CO₂ uptake per leaf area than their C3 and C4 counterparts [39]. Their greater water use efficiency makes CAM plants a potentially important contributor to sustainable agriculture in arid and semiarid regions of the world. While the modelling of CAM is still in its infancy

[45, 34, 4, 5, 6, 47], a comprehensive and faithful model of CAM photosynthesis coupled to local climate conditions would allow for more accurate estimates of CAM productivity and the associated crop yield in arid and semiarid regions. A recent model [2] attempts to couple CAM photosynthesis to climatic conditions. Here we explore this model to better understand the resulting carbon assimilation predictions under conditions of external environmental forcing.

The modeling of CAM is challenging because the exact functioning of CAM is not completely known. Nevertheless the model of [2] moves forward based on the assumption that the CAM circadian rhythm functions through a combination of mechanisms, including the regulation of enzymes such as RuBisCO and PEPC, gene expression, the cytoplasmic pH and vacuolar malic acid levels, and the state of the cell vacuole membrane [32]. The overall effect of these mechanisms is assumed to produce a relaxation oscillation consisting of a slow accumulation or release of malic acid followed by a more rapid change in the overall state of the plant (enzyme levels, gene expression, the cytoplasmic pH and vacuolar malic acid levels, etc.). Such oscillations are ubiquitous in biology, especially in descriptions of circadian rhythms. Indeed, archetypical relaxation oscillators such as the Van der Pol system have been used to analyze the circadian rhythm of the human core temperature as well as contractions of the human heart [7, 49]. The circadian rhythm oscillator model formulated in [2] consists of two nonlinear differential equations, the first of which describes the overall circadian state of the plant and the second of which includes a photosynthesis model for the influx and efflux of carbon (CO_2) from the cell vacuole of the CAM plant. This new system, while affected by changes in external conditions due to dependencies on light, leaf temperature, and leaf potential, also reproduces the endogenous rhythm of CAM that persists in continuous light conditions [36, 56]. This work aims to understand the new system under constant and periodic environmental forcings and to better understand the rates of carbon assimilation predicted by the model.

After a brief overview of CAM, Section 2 presents an outline of the coupled system of equations, one describing the circadian state of the system and the other describing the malic acid concentration in the vacuole, which together determine the circadian rhythm. We then

analyze the behavior of the system under continuous environmental conditions in Section 3 and discuss in Section 4 the effects on the circadian rhythm from periodically forcing certain inputs (irradiance, temperature and leaf potential) to represent day-night cycles. Finally, Section 5 presents the main conclusion. Details of the model formulation are provided in the Appendices.

2. CAM model

CAM plants, such as many cacti, bromeliads, agave, and orchids, are characterized by their ability to conserve water by temporally separating the fixation of carbon and the dark reactions of photosynthesis (the Calvin cycle). Unlike C3 and C4 plants, which immediately pass the stored carbon to the Calvin cycle, CAM plants fix carbon as malic acid at night, when the higher relative humidity reduces evapotranspiration from the plants' stomata. During the day, CAM plants close their stomata, thereby preserving water, and decarboxylate the malic acid to complete the Calvin cycle with energy from the light reactions of photosynthesis [3]. The fluxes of carbon into and within the CAM plant are controlled by its endogenous circadian rhythm [36, 56].

In general, this circadian rhythm may undergo four phases. In Phase I, which occurs at night, stomata open to allow the plant to fix atmospheric CO_2 , which is stored in the vacuole. Phase II consists of a stomatal opening in the morning, when the carbon flux transitions from malic acid fixation to Calvin cycle synthesis. During the day (Phase III), the stomata close and the decarboxylation of malic acid creates an internal increase in CO_2 for the Calvin cycle. Finally, during Phase IV, which occurs during the late afternoon or evening before dusk, the stomata reopen for the net uptake of atmospheric CO_2 for Calvin cycle synthesis [2, 46]. During the onset of water scarcity, Phases II and IV are the first to be suppressed to preserve water from the larger daytime transpiration fluxes created by irradiance and higher temperatures, but all phases may eventually be suppressed as a drought becomes more severe [2, 33, 35, 14]. In addition, facultative CAM plants may switch between C3 and CAM photosynthesis depending on water status. These plants may not undergo Phase I under well-watered conditions [57].

98 2.1 Model structure and balance equations

99 The circadian rhythm of CO₂ uptake and release is modelled following [2], and consists of a
 100 coupled system of nonlinear differential equations for z , which summarizes the global effect
 101 of plant enzyme activity, gene expression, and vacuolar activity, and M , which represents
 102 the concentration of malic acid in the cell vacuole. The first nonlinear differential equation of
 103 the CAM circadian rhythm oscillator is for the overall circadian state of the plant described
 104 by the order variable z and given by

$$t_r \frac{dz}{dt} = \frac{M - M_E(z, T_l)}{M_{max}}, \quad (1)$$

105 where t_r is the relaxation time, which controls the rate of change in z (see Section 3). At low
 106 z values (less than 0.5), malic acid is being stored, while at high z values (greater than 0.5)
 107 malic acid is being released and utilized. The nonlinear function $M_E(z, T_l)$ represents an
 108 equilibrium concentration of malic acid as a function of the circadian state, z , and the leaf
 109 temperature, T_l (see Appendix F). The value of M_{max} is equivalent to the maximum equilib-
 110 rium concentration of malic acid at the low temperature T_L , i.e., $M_{max} = \max[M_E(z, T_L)]$,
 111 and functions to normalize z .

112 The second nonlinear differential equation describes the rate of change in the concentra-
 113 tion of malic acid in the cell vacuole, M , as

$$L_M \frac{dM}{dt} = A_{sv}(M, z, T_l, \psi_l, \phi) + R_{dv}(T_l, \phi) - A_{vc}(M, z, T_l, \phi), \quad (2)$$

114 where L_M is the ratio of malic acid storage volume to the carbon flux surface area and has
 115 units of length. The terms A_{sv} , R_{dv} , and A_{vc} represent the carbon fluxes to and from the
 116 cell vacuole: A_{sv} represents the carbon flux from the stomatal cavity, which is responsible
 117 for exchange of gases with the atmosphere; R_{dv} represents the flux from the dark respiration
 118 node, where breakdown of carbohydrates releases CO₂ to either the Calvin cycle (R_{dc}) or the
 119 cell vacuole (R_{dv}); and A_{vc} represents the flux of carbon to the site of carbon assimilation,
 120 i.e., the Calvin cycle (see Fig. 1 and Appendix A). These functions depend nonlinearly on

the system variables M and z , as well as the external forcing from the leaf temperature, T_l , the leaf water potential, ψ_l , and the light intensity, ϕ .

The carbon flux from the stomata to the cell vacuole is modeled as [2]

$$A_{sv} = (A_{sv,max}(T_l) - R_{dv}(T_l, \phi)) \times f_{\psi_l}(\psi_l) \times f_O(z) \frac{(M_S(T_l) - M)}{(\alpha_2 M_S(T_l) + M_S(T_l) - M)}, \quad (3)$$

where $A_{sv,max}$ represents the overall maximum rate of carbon flux to the cell vacuole (see Appendix E), R_{dv} represents the carbon supplied by dark respiration (see Appendix C), $f_O(z)$ represents the circadian control of the flux (see Appendix G), and $\frac{(M_S(T_l) - M)}{\alpha_2 M_S(T_l) + M_S(T_l) - M}$ is a Michaelis-Menten type term representing the malic acid storage limitation based on the concentration of malic acid that the system can store, M_S (see Appendix H), where α_2 is a parameter between 0 and 1. The function f_{ψ_l} accounts for the effects of water stress, and is dependent on ψ_l , the leaf water potential (see Appendix D).

The carbon flux from the cell vacuole to the carbon cycle is given by

$$A_{vc} = (A_{\phi,c_c,T_l} - R_{dc}(T_l, \phi)) \times (1 - f_O(z)) \frac{M}{\alpha_1 M_{max} + M}, \quad (4)$$

where A_{ϕ,c_c,T_l} represents the CO_2 demand (see Appendix B) and R_{dc} is the flux of carbon from dark respiration to the Calvin cycle (see Appendix C). These functions describe photosynthesis and are dependent on light intensity, mesophyll cytosol CO_2 concentration c_c , and leaf temperature. The mathematical formulation for the Calvin cycle demand, given in Appendices A-C, follows well known photosynthesis models [15, 13]. The order function $1 - f_O(z)$ is a sigmoidal curve ranging from 0 to 1 which describes the relative rate of malic acid diffusion from the cell vacuole to the cytoplasm and the overall activation state of the decarboxylation enzymes, and $\frac{M}{\alpha_1 M_{max} + M}$ is the Michaelis-Menten term representing how the supply of malic acid in the vacuole limits the malic acid diffusion and decarboxylation, where α_1 is a parameter between 0 and 1.

The CAM system can be written as a single second order differential equation for the

143 circadian order, z , in the form of a generalized classical Liénard equation,

$$t_r M_{max} \frac{d^2 z}{dt^2} + M'_E(z, T_l) \frac{dz}{dt} + g\left(\frac{dz}{dt}, z, T_l, \psi_l, \phi\right) = 0, \quad (5)$$

144 where $M'_E(z, T_l) = \frac{d}{dt} M_E(z, T_l)$ [10, 2, p. 130], and

$$g\left(\frac{dz}{dt}, z, T_l, \psi_l, \phi\right) = -\frac{1}{L_M} \left(A_{sv} \left(\frac{dz}{dt}, z, T_l, \psi_l, \phi \right) + R_{dv}(T_l, \phi) - A_{vc} \left(\frac{dz}{dt}, z, T_l, \phi \right) \right). \quad (6)$$

145 The major nonlinearities in the system arise from the circadian order function, $f_O(z)$, in Eqs.
 146 3 and 4, from the Michaelis Menten dependence on M in Eqs. 3 and 4, and from the malic
 147 acid equilibrium equation $M_E(z, T_l)$ in Eq. 1, which is a cubic curve typical of relaxation
 148 oscillations such as the FitzHugo-Nagumo or Van der Pol models. Additive periodic forcing
 149 of the Liénard equation has been described in the literature [50, 10] but this differs from
 150 the more complex forcing induced, in our case, by the environmental variables T_l , ϕ , and ψ_l ,
 151 which act through the multiplicative functions of M'_E and g .

152 2.2 Model Parametrization

153 The model parameters are for *Agave tequilana*, a CAM plant native to Jalisco, Mexico, which
 154 is commonly used in tequila production (see Table 2). Although many of the parameters
 155 are specific to the plant *Agave tequilana*, the main features of the circadian rhythm of other
 156 CAM plant species is expected to be of a similar form and have a similar response to external
 157 parameters.

158 3. Model behavior under constant environmental conditions

159 The model is studied first under constant environmental conditions (constant temperature,
 160 light, and leaf water potential) as a basis for better understanding its response to periodically
 161 forced environmental conditions intended to roughly model the day-night cycle (see Section
 162 4). Under conditions of constant light and temperature considered in this section, the system
 163 may undergo supercritical Hopf bifurcation from a stable fixed point to a limit cycle.

164 3.1 Relaxation oscillations

165 The system oscillates in the (z, M) phase plane around the fixed point that lies at the
 166 intersection of the system nullclines for dM/dt and dz/dt (see Figs. 2 and 3). The nullcline
 167 $dz/dt = 0$ is given by

$$\frac{M}{M_{max}} = \frac{M_E(z, T_l)}{M_{max}} \quad (7)$$

168 and identifies where changes in the system are due only to changes in M (Figs. 2 and 3).
 169 The shape of this nullcline changes depending on the leaf temperature and various nullclines
 170 are displayed between the low leaf temperature of T_L and high leaf temperature of T_H (see
 171 Fig. 2). The nullcline $dM/dt = 0$ identifies where changes in the system are due only to
 172 changes in z and is given by

$$A_{sv}(M, z, T_l, \psi_l, \phi) + R_{dv}(T_l, \phi) - A_{vc}(M, z, T_l, \phi) = 0. \quad (8)$$

173 The shape of this nullcline varies depending on the leaf potential, ψ_l , and two nullclines are
 174 displayed for ψ_{l1} and ψ_{l0} (Fig. 2). An example of an oscillation around the fixed point for
 175 $T_l = 293.15$ K and $\psi_l = -0.5$ MPa is shown in Figure 3e.

176 As is typical of a relaxation oscillation, the system is characterized by two widely sepa-
 177 rated time scales. The longer time scale characterizes the slower filling and emptying of the
 178 cell vacuole with malic acid, while the shorter time scale characterizes the more rapid change
 179 in circadian order and is controlled by the relaxation time, t_r (Eq. 1). For a relatively small
 180 value of t_r , i.e. 1 minute (Fig. 3a), the fast response in dz/dt causes the malic acid concen-
 181 tration to closely follow the thermodynamic equilibrium value defined by the nullcline. An
 182 increase in the relaxation time slows the transition of z and allows a further accumulation
 183 and release of malic acid. As the relaxation time continues to increase to larger values, the
 184 unstable fixed point of the limit cycle transitions to a stable fixed point for the system (Fig.
 185 3b).

186 3.2 Effects of temperature and leaf potential

187 The effect of leaf temperature on the circadian rhythm for non-water stressed conditions
188 (a leaf potential of -0.5 MPa) is shown in Figure 3c, while the effect of leaf potential (at
189 constant temperature 293.15 K) is shown in Figure 3d. As the leaf temperature, T_l , increases
190 past the low temperature, T_L (see Table 1), the circadian system undergoes a supercritical
191 Hopf bifurcation [53]. As the leaf temperature increases further, the oscillations of this limit
192 cycle become smaller until, at a critical temperature, the system trajectory spirals towards a
193 stable fixed point characterized by a moderate to high circadian order, z , and low malic acid
194 concentration (an empty cell vacuole). This transition occurs when the slope of the nullcline
195 (for $dz/dt = 0$) changes from a positive value to a negative value. A limit cycle with the
196 corresponding nullclines, fixed point, and vector field is displayed in Figure 3e.

197 The hydrologic cycle also influences these circadian oscillations through the leaf potential
198 parameter (Fig. 3d). Leaf moisture potential, ψ_l , is dependent on soil moisture and high
199 negative values (e.g., in this paper near -3.0 MPa) are typically considered to be an indicator
200 of plant water stress under drought conditions. As the level of water stress increases, i.e.,
201 the leaf potential decreases, the photosynthesis model becomes impaired, so the oscillations
202 of the system become smaller. As a result, the maximum value of malic acid concentration
203 decreases, narrowing the limit cycle (Fig. 3d). This decrease happens rapidly around the
204 leaf potential value of $\psi_l = -3$ MPa, which is the threshold below which the maximum plant
205 water stress occurs in the model (see ψ_{l_1} in Table 1).

206 3.3 Bifurcations, period length, and carbon assimilation

207 Having noted how temperature and leaf potential impact the circadian rhythm cycle, we now
208 consider the overall behavior of the circadian oscillation and carbon assimilation in response
209 to a range of temperatures and leaf potentials (Fig. 4). At very low leaf temperatures (below
210 283.15 K), there is a fixed point which corresponds to a state with a low value of circadian
211 order and a full vacuole (Fig. 4b). At 283.15 K, the system undergoes a supercritical Hopf
212 bifurcation to a stable limit cycle. As the temperature increases from 283.15 K, the period

length decreases and the oscillations of z become increasingly closer together (Fig. 4b). Finally, after the leaf temperature increases past 300.5 K, there is a second Hopf bifurcation and the system goes to a stable fixed point (Fig. 4b) where M is at a low value and z is at a moderate value. Between 300.5-302.15 K (the value of T_H), CO_2 is both entering and leaving the vacuole, with a very small amount of malic acid being maintained in the vacuole. This temperature (300.5 K) marks the beginning of the breakdown of the CAM cycle under constant light and temperature conditions (as we will describe later, this type of fixed point is unique to constant conditions and disappears under very small forcings of either light or temperature). Finally, at $T_H = 302.15$ K, the stable fixed point transitions from a moderate to a high value of circadian order, z . Here, the vacuole empties completely and carbon flux to the vacuole ceases (Fig. 4c). The location of the bifurcation points agree well with previous results that show the maintenance of the CAM circadian rhythm over the range $T_L = 283.15$ K to $T_H = 302.15$ K [18, 9, 12, 41].

The model parameters for this study are consistent for average nocturnal and diurnal temperatures of 293.15 K and 303.15 K, respectively, and within this temperature range a model period of around 24 hrs. is found (Fig. 4a). The period length around 24 hours is consistent with studies of other circadian rhythms which find that under conditions with no external cues, the free-running period is generally close to, but not necessarily equal to, 24 hours [8, 48]. In this model, as the temperature increases and the oscillations of z become smaller, the period length of the CAM cycle decreases and the oscillations become more rapid (Fig. 4a). At a low temperature of 283 K, the period length is around 60 hours, while at a high temperature of 302 K the period is less than 10 hours. It is remarkable that within this temperature range (283-302 K) a period length of 24 hrs. is obtained naturally (i.e., without calibration) by the complex model proposed in [2] using parameter values obtained from published data.

Though a period of around 24 hrs. was found for the average nocturnal and diurnal temperatures of 293.15 K and 303.15 K, respectively, this period will vary as the average temperature range changes. This occurs in part because CAM plants acclimate to the prevailing average environmental conditions, and they have significant differences in the

242 rates of nocturnal carbon uptake as represented by $A_{sv,max}(T_l)$ and diurnal carbon uptake
 243 for direct carbon assimilation as represented by A_{ϕ,c_i,T_l} for different average temperatures [2,
 244 p. 122]. We account for such acclimation with an exponential increase/decline in $A_{sv,max}$
 245 and A_{ϕ,c_i,T_l} given by the factor

$$f_a(T_{l,avg}) = b_1 \exp(-b_2(T_{l,avg} - T_L)), \quad (9)$$

246 where $T_{l,avg}$ is the average daily temperature over multiple days, T_L represents the low end
 247 of the temperature range of CAM (Table 1), and b_1 and b_2 are parameters specific to the
 248 experimental data of a certain plant species parameters chosen to fit the data (Table 1).
 249 For the constant temperature conditions explored here, $T_{l,avg}$ is simply the constant leaf
 250 temperature T_l . Available experimental data for the period of CAM is for plants that are
 251 acclimated to certain average nocturnal and diurnal temperatures. To compare the period of
 252 the model to this data, we use a slightly increased temperature range of CAM, so the values
 253 of T_L and T_H for this comparison are 278 K and 304 K, respectively. This experimental data
 254 describes an increase in period length with an increase in temperature [56, 18], and based on
 255 the acclimation function, $f_a(T_{l,avg})$, the period of the model agrees well with the data (Fig.
 256 5).

257 The advantage of CAM is the water efficient productivity from the carbon flux A_{sv} , which
 258 is a measure of the amount of carbon taken up and fixed as malic acid at night when water
 259 losses are typically lower (Fig. 4c, f). Note that this flux is only a portion of the total
 260 carbon assimilation and does not include the less water efficient direct carbon assimilation
 261 fluxes of C3 photosynthesis that primarily occur during Phases II and IV. As expected, the
 262 flux A_{sv} is 0 at the stable fixed point, which occurs at low temperatures below 283.15 K,
 263 but when T_l increases past 283.15 K, the assimilation flux rapidly increases to values of
 264 about 200 mmol/m²/day, reaching a maximum value of 220 mmol/m²/day at 293 K. As the
 265 temperature increases and moves out of this optimal range, the assimilation decreases. A
 266 sharp spike appears at 301 K, which corresponds to the breakdown of the CAM cycle when
 267 the system transitions to a stable fixed point between the 300.5-302.5 K (Fig. 4b). This fixed

point, which occurs only under conditions of no forcing or very little forcing, arises when malic acid is simultaneously being taken up and fixed, and is a transitional state before the model terminates the CAM cycle.

Variations in the leaf potential between the allowed values of -3.0 and -0.5 MPa have an equally important effect on the period length and associated carbon assimilation, but a smaller effect on the amplitude of the oscillations (Fig. 4d-f). As the leaf potential becomes less negative, i.e., water stress decreases, the values of the circadian order, z , shift upward slightly, but a similar limit cycle is maintained for all leaf potential values greater than -3.0 MPa (Fig. 4e). The free-running period is, however, greatly affected by changes in the leaf potential, and may vary from 20 to upwards of 80 hours in an almost exponential manner (Fig. 4d). Although no experimental data are available on the effect of leaf moisture potential on CAM period length, the carbon assimilation responds to changes in leaf moisture in an expected manner; as ψ_l approaches the maximum allowed water stress, the flux A_{sv} decreases and the carbon assimilation through the CAM pathway approaches 0, thereby increasing the period length (Fig. 4f). It is noteworthy that, again, an endogenous circadian oscillation centered around 24 hours is obtained using estimated parameters without any calibration; this result lends support that the various complex parameters are faithfully modelled and coupled in a realistic way.

4. Effects of environmental periodic forcing

The behavior of the system is further examined with periodic forcings of temperature and light to simulate the behavior of CAM plants under the natural daytime and nighttime fluctuations observed in the environment. Here, we choose a simplistic description for the temperature, modelling it as a sine wave which increases to a maximum value at noon and decreases to a minimum value at midnight, i.e.,

$$T_l(t) = T_{l,av} + A \sin(\omega t - \pi/2), \quad (10)$$

where A (K) represents the displacement of the temperature from the mean (T_{lav}), ω is $2\pi/p$, $p = 24$ hours, and t is time (hrs). While there are various methods of interpolating temperature data from maximum and minimum daily values, any one of which could easily be coupled to this model [51], this equation is one of the simplest approximations, which has previously been used with respect to plant productivity [58]. The solar radiation is explored using a piecewise function alternating between 0 at night and a parabola during the day [27], i.e.,

$$\phi(t) = \begin{cases} 0 & \text{for } t \bmod 24 < 12 \\ \frac{\phi_h}{36}(-(t \bmod 24)^2 + 24(t \bmod 24) - 648) & \text{for } t \bmod 24 \geq 12, \end{cases} \quad (11)$$

where $\phi(t)$ is the solar radiation (W/m^2), ϕ_h is the maximum solar radiation, ω is $2\pi/p$, $p = 24$ hours, and t is time (hrs). Responses typical to laboratory conditions, i.e. 12 hours of light and 12 hours of darkness, were also explored. The results were found to vary qualitatively from the more realistic light forcing results shown here, suggesting that laboratory experiments which analyze CAM circadian rhythms using such conditions may not be the best predictors for behavior under natural conditions.

4.1 Bifurcations

Forcing the leaf temperature to simulate typical daily conditions has a strong effect on the CAM cycle (Fig. 6). When there is a small degree of temperature forcing, the periodic limit cycle at 300 K becomes unstable and quasi-periodic or chaotic behavior is observed for forcing amplitudes less than 2.7 K. At 2.7 K, the system returns to periodicity, but this time with a much larger amplitude (Fig. 6a). Behavior for forcing amplitudes between 1 and 2.5 K are shown in the inset in Figure 7a, and the transition to chaos or quasiperiodicity observed for $A \leq 1\text{K}$ seems to arise through the period doubling mechanism. When leaf temperature is forced with an amplitude of 1 K, which generates a 2 K range between the maximum and minimum temperatures (Fig. 6c), the response shows many similarities to the unforced version in Figure 4b, but the stable fixed points have disappeared and are replaced by large oscillations (below T_L) and small oscillations (above T_H). Also of note are several windows which appear either chaotic or quasi-periodic. Samples from two of these windows

318 are displayed in Figure 7b and c for more detail. For a leaf temperature amplitude of 5
 319 K (Fig. 6e), an average temperature between 286 and 288.5 K results in quasi-periodic or
 320 chaotic behavior where there seems to be some period doubling, but the system then returns
 321 to a periodic oscillation for higher temperature values, except for a brief transition at 301
 322 to 302 K. On the other hand, a leaf temperature amplitude of 10 K (Fig. 6g), as would
 323 be seen in a typical desert environment, results in a periodic solution for the entire range
 324 of average temperatures between 283 and 302 K. As the average temperature increases past
 325 285 K, there is a jump in the solution and z shifts to lower values, which occurs due to
 326 the changing rates of malic acid uptake ($A_{sv,max}(T_l)$) and release (A_{ϕ,ci,T_l}) that both have
 327 a leaf temperature dependency. Because, under this forcing regime, the temperatures dip
 328 significantly below T_L , z becomes slightly negative in some instances; however, this behavior
 329 is inconsequential as the state of the system is determined by whether z is greater than or
 330 less than 0.5, as set by the order function, $f_O(z)$. Overall, when the temperature is varied
 331 significantly, the amplitude of the resulting oscillations of z becomes nearly the same over
 332 a similar range of average temperatures, unlike the behavior seen when the temperature is
 333 held constant or varied only slightly. This similarity in the oscillation of z leads to a more
 334 consistent carbon assimilation over a wider range of average temperatures.

335 This response to changes in temperature has an important effect on CO₂ assimilation
 336 via the CAM pathway, and interesting behavior begins to emerge when the amplitude of
 337 leaf temperature fluctuations allows the temperature to cross the bifurcation value of 300.5
 338 K during the course of the day (Fig. 6b). For small A less than 2 K, the leaf temperature
 339 remains near 300 K in a range of temperature where carbon assimilation is optimal (Fig.
 340 4c). As soon as A becomes larger than 2K, however, the temperature jumps into the range
 341 where the CAM cycle shuts down (Fig. 4c). Consequently, there is a dramatic decrease in
 342 the average carbon assimilation (Fig. 6b). However, as the amplitude increases further past
 343 2 K, the carbon assimilation improves, reaching moderate values by $A = 15K$. For $A > 2K$,
 344 the last traces of the stable fixed point disappear, and carbon assimilation via the CAM
 345 pathway is maintained for the entire range of average temperatures up to $T_l = 305K$. This
 346 behavior suggests that, under conditions of high mean temperatures, a larger temperature

range, such as that observed in most arid environments, is beneficial for CAM functioning. Such a conclusion is supported by experimental studies which have found that the optimal nighttime temperature is 10 to 20 C, while the optimal daytime temperature is above 20 C [17].

Periodic forcing of the irradiance also has a significant effect on the system's functioning, which is shown here for different temperature inputs, i.e., constant and forced (Fig. 8). Varying the maximum light input, ϕ_h , has essentially no effect when ϕ_h is above the threshold value of 100 W/m², where the leaf temperature and leaf potential are the controlling factors. Since typical maximum light values, ϕ_h , are much greater than this threshold, the response of the plant under a periodically forced light input depends much more on the leaf temperature and water potential than it does on the irradiance. For a maximum light input at a typical environmental value of 500 W/m² and a nighttime light input of 0 W/m², the system exhibits possible chaotic behavior for constant temperatures below about 291.2 K. It then maintains a periodic solution for constant temperatures above this threshold (Fig. 8a). On the other hand, forcing both the light and temperature simultaneously (with the temperature amplitude equal to 5 K), as in Figure 8c, results in mainly periodic solutions. As in the case with temperature forcing, both stable fixed points become unstable when the light is varied diurnally.

Unlike temperature forcing, irradiance forcing alone does not extend the range of mean temperatures under which CAM photosynthesis is expected to function (Fig. 8b). In fact, when compared with the unforced system, the forced system is expected to stop functioning at a slightly lower temperature (301.5 K compared with 302.15 K). This behaviour occurs because the periodic cessation of light does not allow for the fixed point that existed under constant irradiance and between the leaf temperatures of 300.5 to 302.15 K, which was not a realistic state. When the solar radiation is forced diurnally, a clear optimal temperature emerges around 291 K (Figs. 8b, d). This optimal temperature agrees with observed ranges of optimal temperatures for CAM photosynthesis (P. Inglese, personal communication). Although the maximum assimilation is lessened when temperature forcing is added to the picture (Fig. 8d), the optimal average temperature remains the same. The fact that the op-

376 timal temperatures in the model agree with observed ranges suggests that the model may be
377 used to predict optimal combinations of environmental conditions in different environments.

378 As expected, forcing of the temperature, light, or both, always results in entrainment
379 of the CAM cycle to a 24 hour cycle in regions where the response is periodic. This phase
380 locking is an important characteristic of circadian rhythms. Such rhythms are known to
381 deviate from a 24-hour period in the absence of a light-dark cycle, but are entrained by
382 external forcing of environmental conditions, as long as this forcing occurs with a period
383 close to 24 hours [48].

384 4.2 Poincaré section and spectral analysis

385 Poincaré sections and spectral analysis can both be used to distinguish between periodic
386 and chaotic systems. The Poincaré section for a periodically driven system can be obtained
387 by sampling the 3D phase portrait at periodic intervals and shows the instances where the
388 trajectory crosses a plane in the phase space from one direction [1]. Another method of
389 distinguishing between periodicity (or quasi-periodicity) and chaos involves computing the
390 fast Fourier transform (FFT) of the time series. The FFT of a periodic system will show
391 a small number of frequencies above a certain cutoff frequency, while the FFT of a chaotic
392 system will show a much larger number of frequencies above the cutoff value [55].

393 Figures 9a and 9b show a time series and 3D phase portrait, respectively, in conditions
394 of periodic temperature forcing with an average temperature of 298 K and an amplitude of 1
395 K. The time series shows the evolution of the system circadian order with time while the 3-D
396 phase portrait shows the evolution in time of z , M , and T_l . Unlike the regular oscillations
397 which occur under conditions of constant temperature, this example exhibits quasi-periodic
398 behavior. This conclusion is supported by the Poincaré section in Figure 9c, which shows a
399 closed curve, and the spectral analysis of the time series (Fig. 9d), which yields a relatively
400 small number of peaks.

401 Not only is irregular behavior observed when the leaf temperature is forced, but it is
402 also observed when the light is forced, for certain ranges of temperature. For example,

403 forcing the light in the manner described in Section 4 results in a possible chaotic solution
404 when the temperature is held constant at $T_l = 285K$ (Fig. 9e, f), which is supported by
405 the Poincaré section which shows two disjoint curves (Fig. 9g) reminiscent of the Poincaré
406 section obtained from other chaotic attractors such as the Lorenz attractor [11]. The spectral
407 analysis, which shows a less distinctive pattern of frequencies, also supports this possibility
408 (Fig. 9h). It is interesting to note that, while the development of chaos disrupts the plants’
409 functioning significantly, it still undergoes an average of one oscillation every 24 hours (Fig.
410 9e).

411 5. Discussion and Conclusion

412 The model presented in [2], which for the first time fully combines a CAM carbon assimilation
413 model [15] with a model for the circadian dynamical system, allows a realistic coupling of
414 CAM photosynthesis to changing environmental conditions [2], showing how the endogenous
415 circadian rhythm of CAM can be entrained by environmental forcing and how different types
416 of forcing can lead to qualitatively different responses in the temporal behavior and carbon
417 assimilation of CAM plants. Under certain circumstances, the nonlinearities and feedbacks
418 between water status, energy input, and the circadian rhythm of the model give rise to quasi-
419 periodic or chaotic behavior. While the observation of complex temporal dynamics, including
420 chaos, in CAM plants is not new [34, 23], the results given here provide a more detailed and
421 realistic analysis of the conditions under which complex behavior can be expected to occur
422 and what effect this type of behavior may have on plant productivity. They are also the first
423 to show quasi-periodicity in a model of CAM plants.

424 Although periodic forcing of the Liénard’s equation has been mathematically explored
425 in the literature [50, 10], it has typically been done through the additive forcing provided by
426 the introduction of a fourth term, $p(t)$, to the general equation, i.e.,

$$\frac{d^2}{dt^2}(x) + f(x)\frac{d}{dt}x + g(x) = p(t), \quad (12)$$

427 where $p(t)$ is a periodic function of a given frequency. In this general case, it has been

found that there exist stable and unstable periodic solutions with the resulting period equal to that of the forcing term [50]. On the other hand, the periodic forcing of our system (see Eq. 5) appears in a more complex form through the parameters of leaf temperature T_l , which appears in the terms $f(x)$ and $g(x)$, and solar radiation ϕ , which appears in $g(x)$. Consequently this forcing introduces a more complex response which results in the observed quasi-periodic and chaotic behaviors (see Fig. 9). These responses arise due to nonlinearities of the model, and as would be expected, changes in the leaf temperature in three key nonlinear terms (the malic acid equilibrium expression $M_E(z, T_l)$ and the carbon fluxes $A_{sv}(M, z, T_l, \psi_l, \phi)$ and $A_{vc}(M, z, T_l, \phi)$), and changes in the solar radiation in the one key nonlinear term (the carbon flux $A_{vc}(M, z, T_l, \phi)$) both result in possible chaos and/or quasiperiodicity. Despite these key differences, the forced system explored in this paper takes on the period of the forcing term(s) as in the general case of Eq. 12 [50]. Thus the model is always synchronized with the daily diurnal/nocturnal cycle, as would be expected for a relaxation oscillation.

Quasi-periodic and chaotic behaviors are not uncommon in biology and have been previously observed in the circadian rhythms of both plants and animals [25, 23]. In certain situations such behaviors may, in fact, function to increase robustness under varied environmental conditions by allowing for greater flexibility, as has been proposed by Lloyd (1997). Indeed, our model results suggest that CAM plants are able to maintain a relatively high level of carbon assimilation under conditions which cause a chaotic or quasi-periodic response. While the periodic forcing of light and/or temperature encourages irregular responses, it also extends the temperature range over which carbon assimilation will occur via the CAM pathway. Under constant environmental conditions, malic acid will diffuse from the vacuole and the CAM system will stall at temperatures above about 300 K. Under forced environmental conditions, while the behavior may become more erratic, the plant is able to continue assimilation via CAM for the entire temperature range of 283 to 302 K.

The emergence of either quasi-periodicity or chaos often causes changes in the main frequency of oscillation, but it does allow CAM photosynthesis to continue with some regularity, with the time between peaks in the circadian rhythm order ranging from about 24

457 to 30 hours. Overall, the transitions to quasi-periodicity or chaos, which are reversible with
458 small changes in light or temperature [18], appear relatively infrequently under typical en-
459 vironmental conditions and still allow CAM plants to maintain an adequate level of carbon
460 assimilation.

461 In summary, the results presented here demonstrate the sensitivity of CAM plants to
462 external forcing and show how certain combinations of environmental conditions can lead
463 to stable fixed points, rhythmic oscillations, and possible chaotic behavior. Like earlier
464 models of CAM, the model explored here has a dependency on leaf temperature, which is a
465 controlling parameter in the stability of the circadian oscillations of z and M . It also shows
466 a dependency on water availability, with more negative leaf potentials inhibiting carbon
467 assimilation and less negative leaf potentials increasing assimilation. A CAM cycle period of
468 24 hours is found under constant environmental conditions. However, when an acclimation
469 factor adjusts the carbon fluxes for the average temperature found over multiple days, the
470 period of CAM measured experimentally [56, 19, 4] matches the model period for a range of
471 temperatures. This acclimation factor suggests that studies of CAM productivity should
472 account for the change in the average daily temperature that occurs over the course of a
473 growing season. While changing the magnitude of a constant level of light has little to no
474 effect on the circadian rhythm of the model, periodic forcings of light and temperature may,
475 in some cases, lead to quasi-periodicity and/or chaos, but more importantly such forcings
476 extend the range of temperature under which assimilation is expected to occur. In addition,
477 the predicted behavior of this CAM model as a function of temperature agrees well with
478 experimental studies.

479 While moisture conditions in this work were considered indirectly through the leaf poten-
480 tial, it would be interesting to also couple this CAM model with the soil-plant-atmosphere
481 continuum model component of [2] and introduce forcing with stochastic rainfall to inves-
482 tigate the hardiness of CAM plants in environments with varying water availabilities. This
483 would allow an exploration of carbon vs. water starvation in CAM plants and estimates of
484 CAM productivity for particular species in a given area.

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493 Appendix

494 In the following appendices, we provide brief descriptions of the model components first
495 presented in [2].

496 A. Mass carbon balance

497 As per [2], the net influx of CO₂ into the plant cytosol from the stomata, A_n , is assumed to
498 be in equilibrium with the outgoing CO₂ fluxes, A_{sc} to the Calvin cycle and A_{sv} to the cell
499 vacuole, i.e.,

$$A_n = A_{sc} + A_{sv}. \quad (\text{A.1})$$

500 The dark respiration flux, R_d , from the mitochondria, consists of two components, R_{dc} , the
501 flux emitted to the Calvin cycle, and R_{dv} , the flux to the cell vacuole, i.e.,

$$R_d = R_{dc} + R_{dv}. \quad (\text{A.2})$$

502 At the Calvin cycle site, the carbon flux, A_c , is supplied by the dark respiration as R_{dc} , by
503 the stomatal cavity as A_{sc} , or by the cell vacuole as A_{vc} , i.e.,

$$A_c = A_{sc} + A_{vc} + R_{dc}. \quad (\text{A.3})$$

504 The CO₂ influx A_n is supplied from the atmosphere as steady-state Fickian diffusion with
 505 diffusion coefficient $g_{ms,CO_2} = \frac{g_{m,CO_2}g_{s,CO_2}}{(g_{m,CO_2}+g_{s,CO_2})}$ (the series of the mesophyll and stomatal con-
 506 ductance) as

$$A_n = g_{ms,CO_2}(c_s - c_i), \quad (\text{A.4})$$

507 where c_s is the atmospheric concentration of CO₂ and c_i is the cytosol concentration of CO₂.
 508 From Equation A.4, g_{ms,CO_2} can be expressed as

$$g_{ms,CO_2} = \frac{A_n}{c_s(1 - R)} \quad (\text{A.5})$$

509 where R is the ratio of mesophyll to atmospheric CO₂ concentration [41, 42]. Following [30],
 510 R is expressed as a function of vapor pressure deficit, D , as

$$R = 1 - \frac{1}{a_1} \left(1 + \frac{D}{D_x} \right) \quad (\text{A.6})$$

511 with empirical constants a_1 and D_x .

512 B. Carbon assimilation model

513 As per [2], the net photosynthetic demand for CO₂ from the cell vacuole, A_{vc} , is modeled
 514 after Farquhar et. al. [15] and is expressed as a function of solar radiation, ϕ (W/m²),
 515 mesophyll CO₂ concentration, c_c (ppm), and the leaf temperature T_l (K), i.e.,

$$A_{\phi,c_i,T_l} = f(\phi, c_c, T_l) = \min(A_c, A_Q), \quad (\text{B.1})$$

516 where A_c , the Rubisco-limited photosynthesis, is modeled as

$$A_C = V_{c,max}(T_l) \frac{c_c - \Gamma^*}{c_c + K_c(1 + o_i/K_o)}, \quad (\text{B.2})$$

517 and A_Q , the electron transport-limited photosynthesis, is modeled as

$$A_Q = \frac{J(c_c - \Gamma^*)}{4(c_c + 2\Gamma^*)}, \quad (\text{B.3})$$

518 where $V_{max}(T_l)$ is the maximum carboxylation rate, K_c and K_o are the Michaelis-Menten
 519 coefficients for CO_2 and O_2 , respectively, and Γ^* is the CO_2 compensation point. For the
 520 Phase III flux A_{vc} (Eq. 4), notice that the C3 demand A_{ϕ, c_c, T_l} is no longer driven by the
 521 lower concentration c_i of Phases II and IV but instead is driven by the larger concentration c_c
 522 because of the increase in the mesophyll cytosol CO_2 from the Phase III decarboxylation of
 523 malic acid [2, p. 117]. In A_Q , J is the electron transport rate and is equal to $\min(J_{max}(T_l), Q)$
 524 where Q is the amount of solar radiation ϕ (in W/m^2) converted to mol electrons ($\text{M}^{-2}\text{s}^{-1}$).
 525 Q is given by

$$Q(\phi) = \frac{\phi \lambda}{N_a h c} \times 0.5 \times \kappa_2, \quad (\text{B.4})$$

526 where 50 percent of the incoming radiation is considered photosynthetically active radiation
 527 (PAR) [21], λ is the average wavelength (m) for PAR (assumed to be 550 nm), h is Planck's
 528 constant (Js), c is the speed of light (m/s), N_a is Avogadro's constant (mol^{-1}), and κ_2 is
 529 the quantum yield of photosynthesis in mol CO_2 mol^{-1} photons (assumed to be 0.3 [31, 2]).
 530 $J_{max}(T_l)$ is given by

$$J_{max}(T_l) = J_{max0} \frac{\exp[\frac{H_{vj}}{RT_0}(1 - \frac{T_0}{T_l})]}{1 + \exp(\frac{S_{vj}T_l - H_{dj}}{RT_l})}. \quad (\text{B.5})$$

531 The maximum carboxylation rate, $V_{c,max}$, and the CO_2 compensation point, Γ^* , are given
 532 by

$$V_{c,max}(T_l) = V_{c,max0} \frac{\exp[\frac{H_{vV}}{RT_0}(1 - \frac{T_0}{T_l})]}{1 + \exp(\frac{S_{vV}T_l - H_{dV}}{RT_l})} \quad (\text{B.6})$$

533 and

$$\Gamma^*(T_l) = \gamma_0[1 + \gamma_1(T_l - T_0) + \gamma_2(T_l - T_0)^2]. \quad (\text{B.7})$$

534 The temperature dependence of the Michaelis-Menten constants K_c and K_o is described by
 535 a modified Arrhenius equation, i.e.,

$$K_x(T_l) = K_{x0} \exp \left[\frac{H_{Kx}}{RT_0} \left(1 - \frac{T_0}{T_l} \right) \right], \quad (\text{B.8})$$

536 where x stands for either c or o . Parameter values can be found in Table 3.

537 C. Dark Respiration CO₂ flux

538 A modified Arrhenius equation also describes the temperature dependence of the flux of
539 carbon from dark respiration [26], i.e.,

$$R_d(T_l) = R_{d0} \exp \left[\frac{H_{Rd}}{RT_0} \left(1 - \frac{T_0}{T_l} \right) \right]. \quad (\text{C.1})$$

540 This flux of carbon is assumed to be redirected to either the Calvin cycle, R_{dc} , or to the
541 cell vacuole R_{dv} . When solar radiation, ϕ , is non-zero, almost all dark respiration CO₂ is
542 directed to the Calvin cycle such that

$$R_{dc}(T_l, \phi) = R_d(T_l)(1 - \exp(-\phi)). \quad (\text{C.2})$$

543 When solar radiation is absent, i.e., $\phi = 0$, we assume that all dark respiration CO₂ is
544 converted to malic acid and transported to the cell vacuole, i.e.,

$$R_{dv}(T_l, \phi) = R_d(T_l) \exp(-\phi). \quad (\text{C.3})$$

545 D. Effect of leaf water potential

546 We assume that the net C uptake for malic acid storage is negatively impacted at leaf water
547 potentials below ψ_{l1} and linearly decreases to zero at ψ_{l0} [13], i.e.,

$$f_{\psi_l}(\psi_l) = \begin{cases} 0 & \psi_l < \psi_{l0} \\ \frac{\psi_l - \psi_{l0}}{\psi_{l1} - \psi_{l0}} & \psi_{l0} \leq \psi_l \leq \psi_{l1} \\ 1 & \psi_{l1} < \psi_l \leq 0. \end{cases} \quad (\text{D.1})$$

548 E. Effect of temperature on nocturnal carbon uptake

549 Based on experimental data [41, 37, 43, 59], we assume that the temperature dependence on
550 the net carbon uptake of Phase I has a parabolic form centered at an optimum temperature,

551 i.e.,

$$A_{sv,max}(T_l) = A_{sv,max0}[1 - k(T_l - T_{opt})^2], \quad (E.1)$$

552 where T_l is the leaf temperature, T_{opt} is the optimal leaf temperature, $A_{sv,max0}$ is the optimum
553 rate of carbon fixation as malic acid, and k is a scale parameter (see [2]).

554 F. Equilibrium value of malic acid

555 The cubic function $M_E(z, T_l)$ represents the equilibrium concentration of vacuolar malic acid
556 for the current circadian order z ,

$$M_E(z, T_l) = M_{max} \left[\left(\frac{T_H - T_l}{T_H - T_L} + 1 \right) c_1 [\beta(z - \mu)]^3 - \frac{T_H - T_l}{T_H - T_L} [\beta(z - \mu) - c_2] \right], \quad (F.1)$$

557 where T_l is the leaf temperature (K), and c_1 , c_2 , β and μ are constants which function
558 to position the curve [2]. $T_H=302.15$ K and $T_L=283.15$ K represent the bounds of the
559 average temperature range over which the circadian rhythm of CAM has been observed
560 [56, 34, 41, 12, 9, 19, 20].

561 G. Order function

562 The order function $f_O(z)$ represents the activity of the enzyme PEPC, the vacuolar H+-
563 ATPase and/or H+-PPase pump for malic acid, and the decarboxylation enzymes for malic
564 acid, which control the creation and release of malic acid. We include the functional depen-
565 dency on z as a switch represented by

$$f_O(z) = \exp \left[- \left(\frac{z}{\mu} \right)^{c_3} \right], \quad (G.1)$$

566 where μ is the position parameter and c_3 controls the slope of $f_O(z)$ [2]. $f_O(z)$ is a sigmoidal
567 curve comprised between 0 and 1, of the type often used as a vulnerability function in plant
568 physiology [52, 54].

569 H. Storage of malic acid

570 The maximum concentration of malic acid that the system is capable of storing is given by

$$M_S(T_l) = M_{max} \left[\frac{T_H - T_l}{T_H - T_L} (1 - \alpha_2) + \alpha_2 \right], \quad (\text{H.1})$$

571 where T_H and T_L are the temperature range values of the circadian rhythm, T_l is the leaf
572 temperature, M_{max} is the maximum malic acid concentration at T_L , and α_2 is a parameter
573 between 0 and 1. At small values of α_2 , the disparity between the maximum equilibrium
574 value, $M_{E,max}(z, T_l)$ and the storage limitation $M_S(T_l)$ is small, and a smaller half reaction
575 constant of $\alpha_2 M_S(T_l)$ is required to temper the malic acid flux to the vacuole near the
576 equilibrium concentration, $M_E(z, T_l)$. When α_2 is large, the disparity between $M_{E,max}(z, T_l)$
577 and $M_S(T_l)$ increases, and a larger half reaction constant is required to temper the malic
578 acid flux to the vacuole near the equilibrium concentration.

Table 1: General model parameters

Parameter	Value	Units	Description
a_1	6.4 ^a		Eq. A.6
D_x	0.0068 ^a	kg kg ⁻¹	Eq. A.6
$\frac{g_{m,CO_2}}{g_{s,CO_2}}$	1 ^b		Mesophyll conductance (unit leaf area)
p_a	1.013·10 ⁵	Pa	Air pressure
λ_w	2.5·10 ⁶	J kg ⁻¹	Latent heat of water vaporization
ρ	1.2	kg m ⁻³	Air density
ψ_{11}	-0.5 ^c	MPa	Eq. D.1
ψ_{l0}	-3 ^c	MPa	Eq. D.1
T_H	302.15 ^d	K	Eq. F.1
T_L	283.15 ^d	K	Eq. F.1
t_r	95	min	Eq. 1, relaxation time
α_1	1/100		Eq. 4, $0 \leq \alpha_1 \leq 1$
α_2	1/7		Eq. H.1, $0 \leq \alpha_2 \leq 1$
k	0.003 ^e		Eq. E.1
T_{opt}	288.65 ^e	K	Eq. E.1
c_a	350	ppm	Atmospheric CO ₂ concentration
c_c	500	ppm	Cytoplasm CO ₂ concentration
b_1	8.3		Eq. 9
b_2	1/7		Eq. 9

^a Average value for C₃ from Table 2 (Lohammer column) in [26].

^b Based on ratio for an arid environment [16].

^c Based on the data of [28] and [38].

^d Based on data discussed in Appendix F.

^e Best fit to the nocturnal carbon uptake data of [41].

Table 2: Model parameters specific to *Agave tequilana*

Parameter	Value	Units	Description
<i>Plant Parameters</i>			
LAI	6 ^a		Leaf Area Index
L_M	2.7 ^b	mm	Malic acid stor. vol. to leaf surf. ratio
$V_{c,max0}$	16.5 ^c	$\mu\text{mol}_{\text{CO}_2} \text{ m}^{-2} \text{ s}^{-1}$	Eq. B.6
M_{max}	126 ^c	mol m^{-3}	Maximum malic acid concentration
$A_{sv,max0}$	11.25 ^c	$\mu\text{mol m}^{-2} \text{ s}^{-1}$	Max. rate for malic acid synthesis
<i>Model Initial Conditions</i>			
z	0.5		Circadian order
M	0	mol m^{-3}	Malic acid concentration

^a [37, 40] on a total leaf area basis (both sides).
^b Based on chlorenchyma thickness (one leaf side) of 3 mm [37, p. 40] adjusted by an intracellular air space of 10-percent [37, p. 41].
^c Based on the carbon flux data of [44] and [24].

Table 3: Parameters for photosynthesis model

Parameter	Value	Units	Description
H_{Kc}	59430 ^a	J mol ⁻¹	Activation energy for K_c
H_{Ko}	36000 ^a	J mol ⁻¹	Activation energy for K_o
H_{Rd}	53000 ^a	J mol ⁻¹	Activation energy for R_d
R_{d0}	0.32 ^a	$\mu\text{mol mol}^{-1}$	Dark respiration at T_0
H_{vV}	72000 ^c	J mol ⁻¹	Activation energy for $V_{c,max}$
H_{dV}	200000 ^c	J mol ⁻¹	Deactivation energy for $V_{c,max}$
J_{max0}	$2V_{c,max0}$ ^b	J mol ⁻¹	Eq. B.5 (see Table 2)
H_{vJ}	50000 ^c	J mol ⁻¹	Activation energy for J_{max}
H_{dJ}	200000 ^c	J mol ⁻¹	Deactivation energy for J_{max}
K_{c0}	302 ^a	$\mu\text{mol mol}^{-1}$	Michaelis constant for CO ₂ at T_0
K_{o0}	256 ^a	mmol mol ⁻¹	Michaelis constant for O ₂ at T_0
o_i	0.209 ^a	mol mol ⁻¹	Oxygen concentration
R	8.31	J mol ⁻¹ K ⁻¹	Universal Gas constant
S_{vV}	649 ^c	J mol ⁻¹	Entropy term for carboxylation
S_{vJ}	646 ^c	J mol ⁻¹	Entropy term for electron transport
T_o	293.2 ^a	K	Reference temperature
γ_o	34.6 ^a	$\mu\text{mol mol}^{-1}$	CO ₂ compensation point at T_0
γ_1	0.0451 ^a	K ⁻¹	Eq. B.7
γ_2	0.000347 ^a	K ⁻²	Eq. B.7

^a Value for C₃ per [26].^b On average J_{max0} is twice $V_{c,max0}$, see [22].^c Average C₃ value per [22].**Table 4:** Circadian oscillator constants

Constant ^a	Value ^b
c_1	0.365
c_2	0.55
c_3	10
μ	0.5
β	2.764

^a see Appendices F and G.^b Based on [2].

Figure 1: Carbon assimilation model schematic. The circadian order variable, z , and malic acid concentration, M , serve as a control variables for the carbon fluxes (arrows).

Figure 2: Nullclines $dz/dt = 0$, shown for different leaf temperatures ($T_L = 283.15$ K to $T_H = 302.15$ K), and $dM/dt = 0$, shown for different leaf potentials ($\psi_{l2} = -2.95$ MPa to $\psi_{l1} = -0.5$ MPa).

Figure 3: Panels a) and b) show phase diagrams of the circadian system for different relaxation time constants, t_r , when leaf potential $\psi_l = -0.5$ MPa and leaf temperature $T_l = 293.15$ K. Panel c) shows effects of leaf temperature, T_l , when $\psi_l = -0.5$ MPa. Panel d) shows effects of leaf potential, ψ_l , when $T_l = 290$ K. Panels e) and f) display vector fields and nullclines for specific phase portraits in panels c) and d). $t_r = 95$ minutes unless otherwise noted.

Figure 4: Panels (a-c) show the period length, bifurcation diagram, and carbon assimilation, respectively, under various leaf temperatures, and panels (d-f) show the same quantities under various leaf potentials. In (a-c), leaf potential is held constant at -0.5 MPa, while in (d-f), leaf temperature is held constant at 293.15 K. In all cases, $t_r = 95$ minutes.

Figure 5: Period length and comparison to experimental data, where circles represent the data from [18, 4] and squares represent the data from [56]. The carbon fluxes are adjusted by an acclimation factor of f_a (see Eq. 9). The leaf potential is held constant at -0.5 MPa, $t_r = 95$ minutes, $T_L = 278$ K, and $T_H = 304$ K.

Figure 6: Bifurcation diagrams and carbon assimilation for conditions of forced temperature. In (a) and (b) the amplitude of forcing is varied with $T_{l,av} = 300$ K. In (c-h) the average temperature is varied with constant amplitude of $A = 1$ K (c,d), $A = 5$ K (e,f), and $A = 10$ K (g,h). Insets from panels (a) and (b) are shown in Fig. 7.

Figure 7: Details of the bifurcation diagrams: (a) is an inset from Fig. 6a and (b),(c) are insets from Fig. 6c.

Figure 8: Bifurcation diagrams and carbon assimilation are shown for light forced as a piecewise quadratic equation, with temperature held constant (a,b), and light forced as a piecewise quadratic equation, with temperature forced with an amplitude of 5 K (c,d). In both scenarios, the maximum light intensity is $\phi_h = 500$ W/m².

Figure 9: Time series, phase portrait, Poincaré section, and Fast Fourier Transform. In (a-d) leaf temperature is forced as a sine wave, with $T_{l,av} = 298$ K and $A = 1$ K. In (e-h) irradiance is forced ($\phi_h = 500$ W/m²) with $T_l = 285$ K.

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Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9