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Sink feedback regulation of photosynthesis in vines: measurements and a model

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Abstract

An experimental and modelling study of source–sink interactions in *Vitis vinifera* L., cv. Cabernet Sauvignon, rooted cuttings under non-limiting environmental conditions with a 12 h photoperiod is presented here. After 4 h, measured photosynthesis, stomatal conductance and leaf carbohydrate content reached maximum values. Over the remainder of the photoperiod, photosynthesis and stomatal conductance decreased continuously, whereas leaf carbohydrate content remained relatively constant. Because the experiment took place in a non-limiting environment, the results suggest that stomatal regulation of photosynthesis was mediated by an internal factor, possibly related to sink activity. A simple 1-source, 2-sink model was developed to examine the extent to which the data could be explained by a hypothetical sink-to-source feedback mechanism mediated by carbohydrate levels in either the mesophyll, the source phloem or the phloem of one of the two sinks. Model simulations reproduced the data well under the hypothesis of a phloem-based feedback signal, although the data were insufficient to elucidate the detailed nature of such a signal. In a sensitivity analysis, the steady-state response of photosynthesis to sink activity was explored and predictions made for the partitioning of photosynthate between the two sinks. The analysis highlights the effectiveness of a phloem-based feedback signal in regulating the balance between source and sink activities. However, other mechanisms for the observed decline in photosynthesis, such as photoinhibition, endogenous circadian rhythms or hydraulic signals in the leaf cannot be excluded. Nevertheless, it is concluded that the phloem-based feedback model developed here may provide a useful working hypothesis for incorporation into plant growth models and for further development and testing.

Key words: *Vitis vinifera* L., photosynthesis, sink, feedback, model.

Introduction

For many plant species, the activities of carbon source and sink organs appear to be closely co-ordinated such that a balance is maintained between the source supply and the sink demand (Wardlaw, 1990; Ho, 1992; Foyer *et al.*, 1995). In vines, for example, defoliation (source limitation) leads to lower grape growth and lower yield (Candolfi-Vasconcelos and Koblet, 1990; Foyer *et al.*, 1995), while removal or thinning of grapes (sink limitation) leads to lower rates of photosynthesis (Iacono *et al.*, 1995; Foyer *et al.*, 1995). Understanding the mechanistic basis of source–sink interactions and their integrated outcome at the whole-plant level is of particular relevance to vines, where practices such as pruning, leaf and shoot removal, and crop load adjustment are commonly performed in order to enhance fruit quality.

Currently, however, the mechanisms underlying sink–source interactions observed at the whole-plant level are not well understood. On the one hand, *in vitro* experiments have demonstrated that mesophyll carbohydrate concentration, which depends on the local balance between assimilation and export, can modify the expression of photosynthetic gene promoters (Koch *et al.*, 1992, 1996; Sheen, 1994; Jang and Sheen, 1994). On the other

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hand, a continuous, diurnal decline in photosynthesis from mid-morning is widely-observed in vines (Downton *et al.*, 1987; Chaves *et al.*, 1987; Chaumont *et al.*, 1994), even under non-limiting environmental conditions (Correia *et al.*, 1990, 1995), suggestive of sink-limitation, and yet leaf sucrose levels do not increase concomitantly but instead remain relatively stable throughout the day (Correia *et al.*, 1990; Chaumont *et al.*, 1994). In the latter case, one possible interpretation is that photosynthetic rates decline in direct response to a build-up of carbohydrate in the plant other than in the mesophyll, including in the sinks themselves, via a currently unidentified biochemical signal.

The objective here is to develop and evaluate a simple mathematical representation of source–sink interactions for eventual use in vine growth models suitable for management applications. Previous authors have developed mechanistic models incorporating the effect of source activity on carbohydrate transport to, and unloading by, competing sinks (Thornley, 1972; Dewar, 1993; Minchin *et al.*, 1993). Here the model of Minchin *et al.*, consisting of one source and two sinks, is extended to incorporate a feedback effect of sink activity on source activity (Minchin *et al.*, 1993). Following Minchin *et al.*, the purpose here is not to build a mechanistic model incorporating all the perceived biochemical detail, but to seek the minimal amount of mechanistic detail required to account for the phenomenon of sink–source interactions observed in whole-plant experiments. This modelling approach is also appropriate from a pragmatic viewpoint, in terms of developing a practical model for addressing management problems.

In view of the above-mentioned uncertainty regarding the nature of the feedback mechanism, three alternative hypotheses are examined in the model, according to which photosynthesis is negatively related to carbohydrate concentration in either the mesophyll, the source phloem or in the phloem of one of the two sinks. In order to provide data to evaluate the model, the diurnal courses of leaf photosynthesis, stomatal conductance and leaf carbohydrate content of a single illuminated source leaf in Cabernet-Sauvignon cuttings were measured. Recalling the modelling objective noted above, the purpose here was simply to examine the extent to which the data could be reproduced by a semi-empirical representation of sink–source feedback interactions, rather than to determine experimentally the precise nature of these interactions in all their detail.

After describing the experimental methods and results, the model is presented and, for each feedback hypothesis, the simulated dynamic behaviour of leaf photosynthesis and carbohydrate content is compared with the experimental data. Finally, the predicted steady-state response of photosynthesis to sink activity is examined, in order to explore the general behaviour of the model, and to generate further model predictions for future testing.

Materials and methods

Data were collected in a controlled environment on eight rooted cuttings of *Vitis vinifera* L. cv. Cabernet-Sauvignon with similar leaf areas, during a 12 h period within the grape maturation phase. Two types of plant were considered, those having fruits and those where the fruits had aborted at an earlier stage of development, in order to assess whether the diurnal course of photosynthesis depends on the nature of the sinks (respectively fruits + roots, and roots only). 150 cm^3 of nutritive solution (Pouget and Delas, 1984) were supplied each day during the entire development stage prior to and including the measurement period. Plants were kept in darkness for 48 h before the measurement period in order to condition the eight plants as uniformly as possible. Comparisons with plants not previously subjected to 48 h of darkness confirmed that the initialization protocol did not affect the subsequent gas exchange results (data not shown).

A single leaf was exposed to constant, saturating light (1200 $\mu mol\ m^{-2}\ s^{-1}$) during a 12 h photoperiod. The rest of the plant was kept in darkness using a cover which permitted gas exchange. Each plant thus consisted of a single source (illuminated leaf) and multiple sinks (fruits + roots, or roots only). This protocol simplified the analysis of carbon assimilation by, and export from, the source. The closed environment of the plants was kept as constant as possible. Temperature, humidity and CO_2 concentration around the sampled leaf were respectively 23.9 ± 1.6 °C, 1.8 ± 0.24 kPa, and 373 ± 10 ppm.

Leaf photosynthesis and stomatal conductance were measured using a portable infrared gas analyser and a Parkinson leaf cuvette (Ciras-1, Broad Automatic Leaf Version, PP systems, Hitchin, Herts, UK). The rate of photosynthetic CO_2 uptake (A) and the intercellular CO_2 concentration (c_i) were calculated (Caemmerer and Farquhar, 1981). Leaf carbohydrate contents were analysed by the enzymatic method on 6 mm diameter foliar discs (Kunst *et al.*, 1984). Carbohydrate export rates were estimated by carbon balance as the rate of net photosynthesis minus the net change in leaf carbohydrate content. CO_2 response curves of photosynthesis were measured after 2, 6 and 10 h of exposure to saturating light. At the end of the photoperiod, pressure chamber measurements of leaf water potentials were made on leaves that were illuminated, shaded and exposed to natural (greenhouse) light (respectively -0.25 ± 0.14, -0.168 ± 0.04 and -0.25 ± 0.07 MPa), and showed no significant differences between illuminated and shaded leaves.

Results

As shown in Fig. 1, the daily time-courses of photosynthesis (A) and stomatal conductance (g_s) were similar for both types of plant sinks (fruits + roots, and roots only). During the growth phase prior to the gas exchange measurements, plants without fruits developed a larger root system (4 g dry weight) than plants with fruits (2 g root dry weight, 4 g fruit dry weight). Thus it appeared over time that the extra root growth in plants without fruits acted as a compensatory sink, to the extent that the diurnal decline in A was independent of the nature of

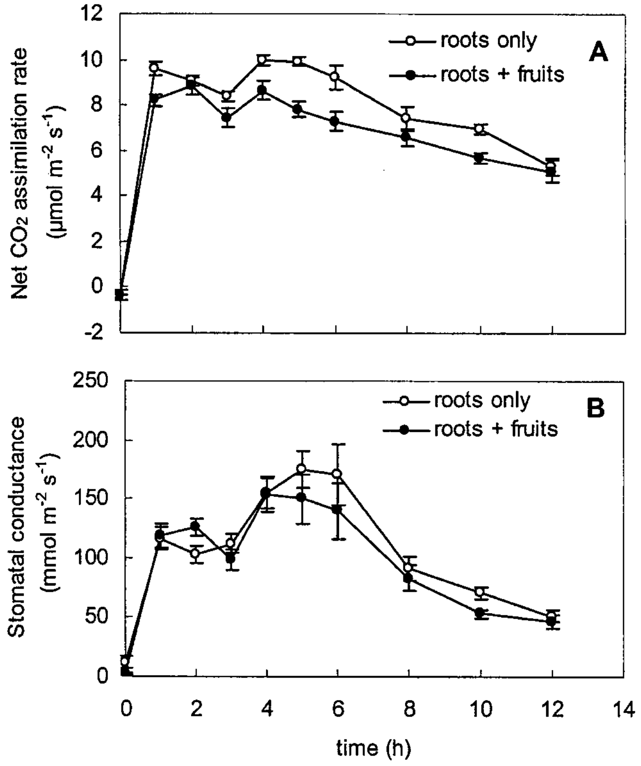

Fig. 1. (A) Leaf CO_2 assimilation and (B) leaf stomatal conductance. Measurements were taken on rooted cuttings of grapevines under saturating light (1200 µmol m^{-2} s^{-1}, 12 h photoperiod) and non-fluctuating environment. Symbols represent the means of four replicates ±s.e. for cuttings with carbon sinks consisting of roots only (○) and fruits+roots (●).

the overall sink. Figure 2A shows the diurnal patterns of A and g_s averaged over all plants, which were used for the model evaluation.

Maximum values of A and g_s were reached after 4 h of continuous, saturating light. Thereafter, A decreased continuously from 9 to 5 µmol m^{-2} s^{-1} during the remainder of the photoperiod, while g_s decreased by two-thirds of its maximum value (Fig. 2A).

Leaf total carbohydrates reached a maximum value coinciding with the maximum rate of photosynthesis, decreased over the next 2 h, but then remained relatively stable during the remainder of the photoperiod (Fig. 2B).

Leaf carbon export rate (E) was calculated by carbon balance as the difference between A and the net change in leaf carbohydrate carbon content. E reached a maximum value after 5 h of light (Fig. 2C), then adjusted to a value close to the rate of carbon assimilation (reflecting the stable value of leaf carbohydrate content).

The initial slope of the A/c_i curve, corresponding to the carboxylation efficiency, did not change significantly during the day, while the CO_2-saturated rate of photosynthesis showed a progressive decline (Fig. 3). However, during the day the operating point was confined to the CO_2-limited portion, so that the observed decline in A could be attributed almost exclusively to the observed decline in g_s.

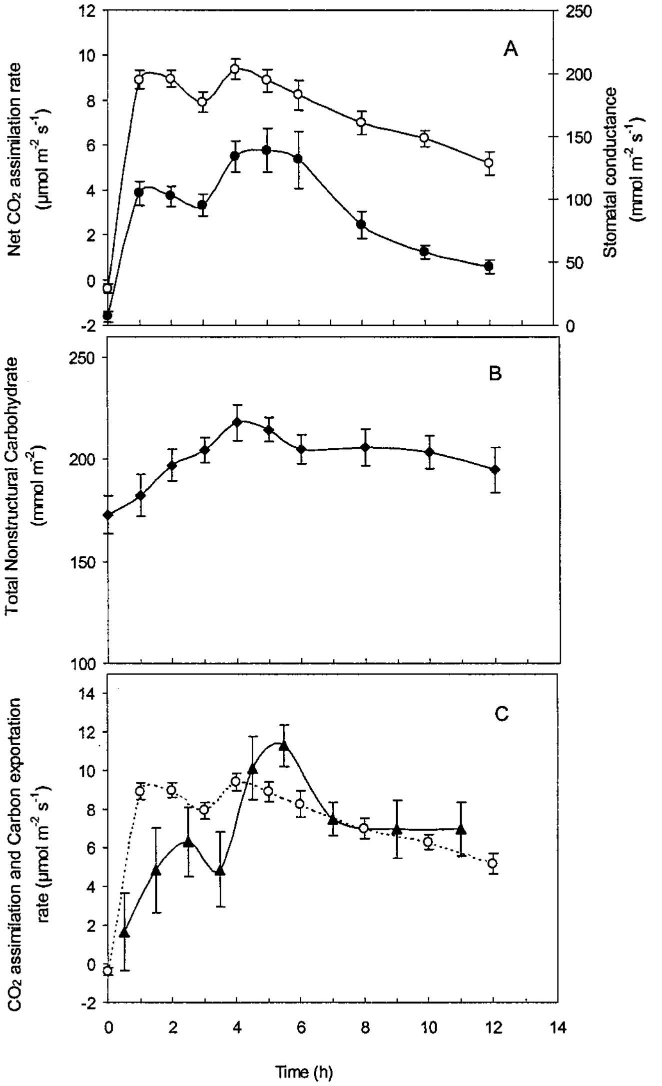

Fig. 2. (A) Leaf CO_2 assimilation (○) and stomatal conductance (●). (B) Leaf total non-structural carbohydrate content (◆). (C) Leaf carbon export rate (▲) and CO_2 assimilation (○). Symbols represent the means of eight replicates ±s.e., obtained by pooling the data from the cuttings with and without fruits in Fig. 1.

The lack of correlation between leaf photosynthesis and total leaf carbohydrate content is similar to that found by other authors (Rodrigues *et al.*, 1993; Chaumont *et al.*, 1994; Correia *et al.*, 1990). The gas exchange results suggest that stomatal regulation of photosynthesis occurs, preventing carbohydrate accumulation in leaves. As the experiment was conducted under controlled, non-limiting environmental conditions, this regulation would appear to be mediated by some internal factor in the plant, one possibility for which might be a build-up of carbohydrate in the plant other than in the source leaf, due to sink limitation. However, as Correia *et al.* noted, while total leaf carbohydrate

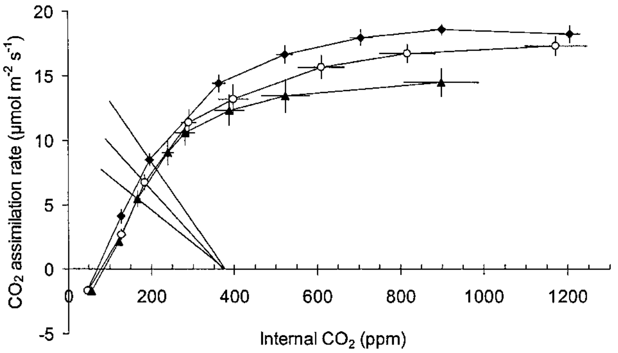

Fig. 3. Responses of net leaf CO_2 assimilation rate (A) to intercellular CO_2 partial pressure (c_i) after 2 (◆), 6 (○) and 10 h (▲) of exposure to saturating light (1250 µmol m^{-2} s^{-1}). Symbols represent the means of eight replicates ±s.e. Straight lines represent the CO_2 supply equation $A = g_s(c_a - c_i)$, where g_s is leaf stomatal conductance and c_a is ambient CO_2 partial pressure (370 ppm), and indicate a progressive decline in assimilation due to stomatal closure.

content remained stable, a feedback role for some component of leaf carbohydrates cannot be discounted (Correia *et al.*, 1990).

In view of the experimental results, and of the overall objective, a simple model was developed to examine further the possibility that the decline in A and g_s is regulated by sink demand. The specific aim was to determine the extent to which the data could be reproduced by a hypothetical carbohydrate-mediated feedback mechanism, either in the mesophyll or elsewhere, and to explore the behaviour of the model more generally through sensitivity analysis in order to generate further testable predictions.

The model

Symbol definitions and units are given in Table 1. Following Minchin *et al.* (Minchin *et al.*, 1993), a model consisting of one source compartment (illuminated leaf) and two sink compartments (e.g. grapes and roots), which are linked by phloem vessels of volume V_0 for the source and V_1, V_2 for the sinks (Fig. 4) is considered. The source is divided further into mesophyll and phloem compartments, in order to examine the possibility that photosynthesis is regulated by one of these components of total leaf carbohydrate content (Correia *et al.*, 1990). There are thus four dynamic carbon pools (mol C): C_m (source mesophyll), C_0 (source phloem), C_1 (sink 1 phloem), and C_2 (sink 2 phloem). The corresponding phloem carbohydrate concentrations (mol C m^{-3} phloem) are given by $s_i = C_i/V_i$ (for i = 0, 1, 2). In the model of Minchin *et al.*, s_0 is a fixed parameter (Minchin *et al.*, 1993); in the present model s_0 is a dynamic variable.

Carbohydrates are assimilated by the source mesophyll during photosynthesis at rate A (as described below in equation 5), and actively loaded into the source phloem at a rate L which depends positively on the mesophyll carbohydrate content. Assuming Michaelis–Menten loading kinetics (Komor, 2000), then

$$L = L_{\max} \times \frac{C_m}{C_m + km_L} \tag{1}$$

where $L_{\max}$ is the maximum loading rate and km_L is a Michaelis constant. As derived previously (Minchin *et al.*, 1993), the transport rates of photosynthate in the branches leading to each sink are given by

$$F_1 = \frac{s_0(\alpha_2 s_{01} + \alpha_0 s_{21})}{\alpha_0(\alpha_1 + \alpha_2) + \alpha_1 \alpha_2} \tag{2a}$$

$$F_2 = \frac{s_0(\alpha_1 s_{02} + \alpha_0 s_{12})}{\alpha_0(\alpha_1 + \alpha_2) + \alpha_1 \alpha_2} \tag{2b}$$

where α_0, α_1 and α_2 are the phloem transport resistances of, respectively, the common pathway and the branch to sinks 1 and 2, and where $s_{ij} = s_i - s_j$ denotes concentration differences. Equations 2a and 2b are based on the Münch hypothesis for flow driven by an osmotically-generated pressure gradient. Substituting the relationships $s_i = C_i/V_i$ (for i = 0, 1, 2) into equation 2 introduces an explicit dependence on the phloem volumes V_i, which can be partially absorbed into the definition of the transport resistances α_i, as described in the Appendix.

The transport rate of photosynthate in the common pathway is then given by

$$F_0 = F_1 + F_2 \tag{3}$$

U_i, the rate of phloem unloading in sink i (i = 1, 2), is described by Michaelis–Menten kinetics with a maximum rate vm_i and Michaelis constant km_i:

$$U_i = vm_i \times \frac{C_i}{C_i + km_i} \tag{4}$$

Three hypotheses are considered for the feedback effect on carbon assimilation, described by a negative dependence of photosynthesis on the carbohydrate pool in either: the source mesophyll C_m (hypothesis 1), the source phloem C_0 (hypothesis 2), or in the phloem of one of the two sinks, taken to be C_1 (hypothesis 3) (Fig. 4). Carbon assimilation is then given by:

$$A = A_{\max} \times \frac{km_A}{C_j + km_A} \tag{5}$$

where j = m, 0 or 1 (respectively, hypotheses 1, 2 and 3), $A_{\max}$ is the maximum rate of carbon assimilation achieved at low carbohydrate levels, and km_A is the value of C_j at which $A = ½\ A_{\max}$. Equation (5) is adopted here as a simple, empirical inhibitory response (Thornley and Johnson, 1990) for which no mechanistic interpretation is assumed.

The rate of change of each pool is then calculated as the difference between the relevant input and

Table 1. *Symbol definitions and units*

Symbol	Definition	Units
C_m	Carbohydrate pool in the source mesophyll	mol C
C_i (i = 0, 1, 2)	Carbohydrate pool in the phloem of the source (i = 0), sink 1 (i = 1) and sink 2 (i = 2)	mol C
V_i (i = 0, 1, 2)	Phloem volume of the source (i = 0) and sinks (i = 1, 2)	m^3 phloem
s_i (i = 0, 1, 2)	Phloem carbohydrate concentrations in the source (i = 0) and sinks (i = 1, 2)	mol C m^{-3} phloem
s_{ij} (i, j = 0, 1, 2)	Carbohydrate concentration difference between pools i and j	mol C m^{-3} phloem
A, A_{max}	Carbon assimilation rate and its maximum	mol C s^{-1}
km_A	Feedback constant	mol C
L, L_{max}	Loading rate and its maximum	mol C s^{-1}
km_L	Michaelis loading constant	mol C
F_i (i = 0, 1, 2)	Assimilate transport rate in the common pathway (i = 0) and in the branches to sinks 1 and 2 (i = 1, 2)	mol C s^{-1}
α_i (i = 0, 1, 2)	Phloem transport resistance in the common pathway (i = 0) and in the branches to sinks 1 and 2 (i = 1, 2)	mol C m^{-6} s
$\tilde{\alpha}_i$ (i = 0, 1, 2)	Rescaled transport resistances (Appendix 1)	mol C s
vm_i (i = 1, 2)	Maximum rate of unloading in sinks 1 and 2	mol C s^{-1}
km_i (i = 1, 2)	Michaelis unloading constant for sinks 1 and 2	mol C
U_i (i = 1, 2)	Phloem unloading rate in sinks 1 and 2	mol C s^{-1}

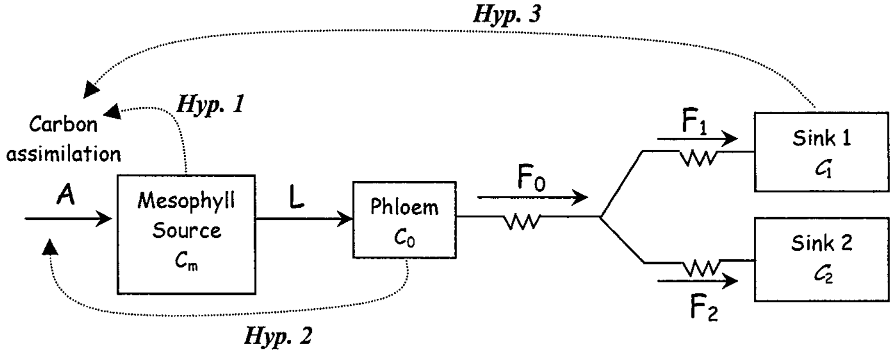

Fig. 4. Schematic representation of the pools and fluxes in the 1-source, 2-sink model. A, carbon assimilation; L, phloem loading; F_0, phloem translocation in the common pathway; F_i (for i = 1, 2), phloem translocation in the branch to sink i. The diagram also indicates the three hypotheses (Hyp. 1–3) considered for the origin of the carbohydrate-mediated feedback signal to A.

output fluxes:

$$\frac{dC_0}{dt} = L - F_0 \tag{6a}$$

$$\frac{dC_1}{dt} = F_1 - U_1 \tag{6b}$$

$$\frac{dC_2}{dt} = F_2 - U_2 \tag{6c}$$

$$\frac{dC_m}{dt} = A - L \tag{6d}$$

Numerical simulations were performed using Euler's integration method with a time step of 10 min.

Evaluation of the three feedback hypotheses

For each feedback hypothesis measured and predicted, values of photosynthesis (A) were compared. Measured total leaf carbohydrate content was compared with the value of $C_m + C_0$ predicted by the model. Model pools and parameter values were therefore expressed on an equivalent per unit leaf area basis. Table 2 (columns 2–4) gives the parameter values and initial pool sizes, corresponding to the best fit for A in each case. An attempt was made to minimize the number of fitted parameters by fixing the phloem unloading parameters and initial values of the carbon pools. In view of the scope of this study, no attempt was made to model the transitory period of stomatal opening and establishment of photosynthesis observed during the first hour of the photoperiod (Fig. 2).

It was impossible to reproduce the data using hypothesis 1, according to which photosynthesis is regulated locally by leaf mesophyll carbohydrate C_m. Under this hypothesis, the predicted diurnal decline in A was always accompanied by a strong increase in total leaf carbohydrate content ($C_m + C_0$), whereas the measured value of leaf carbohydrate content remained relatively stable from $t = 6$ h until the end of the photoperiod, as illustrated in Fig. 5A.

In contrast, under hypotheses 2 (Fig. 5B) and 3 (Fig. 5C), it was possible to find a reasonable fit of the model to the data for both A and $C_m + C_0$, consistent with the conjecture that photosynthesis may respond

Table 2. *Parameters and initial pool sizes used for the dynamic simulations under hypotheses 1–3, and the steady-state sensitivity analysis in Figs 6 and 7 expressed on a per unit leaf area basis; parameter values for hypotheses 1–3 were adjusted to obtain best fits to measured carbon assimilation*

Parameter	Hypothesis 1	Hypothesis 2	Hypothesis 3	Fig. 6A	Fig. 6B	Fig. 7A	Fig. 7B	Units
$A_{\max}$	14	12	12	15	15	varied	12	μmol m^{-2} s^{-1}
km_A	250	300	110	110	300	110	110	mmol C m^{-2}
$L_{\max}$	5	16	25	25	16	25	25	μmol m^{-2} s^{-1}
km_L	20	20	70	70	20	70	70	mmol C m^{-2}
$\tilde{a}_0$	1250	1429	1250	1250	1429	1250	varied	mol m^{-2} s
$\tilde{a}_1$	1667	2000	1667	1667	2000	1667	1667	mol m^{-2} s
$\tilde{a}_2$	1667	2000	1667	1667	2000	1667	1667	mol m^{-2} s
vm_1	10	10	10	10 or varied	10 or varied	10	10	μmol m^{-2} s^{-1}
km_1	200	200	200	200	200	200	200	mmol C m^{-2}
vm_2	10	10	10	10 or varied	10 or varied	20	5	μmol m^{-2} s^{-1}
km_2	200	200	200	200	200	200	200	mmol C m^{-2}
Initial values of the four sugar pools								
$s0_0$	110	110	110	–	–	–	–	mmol C m^{-2}
sm_0	60	60	60	–	–	–	–	mmol C m^{-2}
$s1_0$	20	20	20	–	–	–	–	mmol C m^{-2}
$s2_0$	20	20	20	–	–	–	–	mmol C m^{-2}

to a more distant signal coming either from the source phloem supplying the sinks, or from the sinks themselves. The data are not sufficient to decide between hypotheses 2 and 3.

Steady-state behaviour of the model

A sensitivity analysis of the model was performed, with the aim of exploring more widely the behaviour of the model under hypotheses 2 and 3, and of generating testable predictions that might be used to design future experiments. The simulations presented so far illustrate the transient, dynamic behaviour of the model over a 12 h period, under constant environmental conditions, starting from a given initial condition (Fig. 5). Over a longer period (approximately 100 h), the model eventually reaches a steady state, independent of the initial conditions. Although the steady state is unlikely to be reached under natural conditions, it is useful to explore the sensitivity of the model in the steady state because this eliminates any transient dependence on the initial conditions, thus revealing more clearly the intrinsic properties of the model. (The same approach was adopted by Minchin *et al.*, 1993).

Dependence of source activity on sink demand

Figure 6A shows the steady-state response of photosynthesis (A) to variation in the demand of either sink 1 (vm_1) or sink 2 (vm_2) under hypothesis 3 (feedback signal from sink 1). When the demand of the signalling sink (vm_1) is varied, while that of the non-signalling sink (vm_2) is held fixed, the model predicts that carbon assimilation is stimulated when demand is increased, and suppressed when demand is reduced. Significantly, the same qualitative response occurs when the demand of the non-signalling sink (vm_2) is varied, while that of the signalling sink (vm_1) is held fixed. For example, according to the model, an increase in the sink demand of grapes would lead to a stimulation of photosynthesis, even if it is the roots that generate the feedback signal. Alternatively, according to Fig. 6A, fruit removal from the plant, simulated by decreasing vm_2 and α_2 to zero, would result in a decrease in steady-state carbon assimilation to 5.2 μmol m^{-2} s^{-1}. These results illustrate the interdependence of source and sink carbon pools in the model, mediated by the sink–source feedback mechanism and by linkage through the phloem transport network.

In quantitative terms, the feedback response of assimilation is predicted to be stronger when the demand of the signalling sink is increased, rather than the demand of the non-signalling sink. This difference in feedback response arises from the existence of the phloem resistance in the branch to the non-signalling sink (α_2). When this resistance is set to zero, the effect of varying sink demand on source activity is predicted to be the same for both sinks (data not shown).

Under hypothesis 2 (feedback signal from source phloem), the same qualitative behaviour is found for the feedback effect of sink demand on carbon assimilation (Fig. 6B). Quantitatively, the feedback response of A to varying sink demand is the same regardless of which of the two sinks is modified.

Carbon partitioning to sinks

Following Minchin *et al.*, an investigation of the steady-state dependence of carbon partitioning between the two sinks (F_1 versus F_2) on various model parameters was

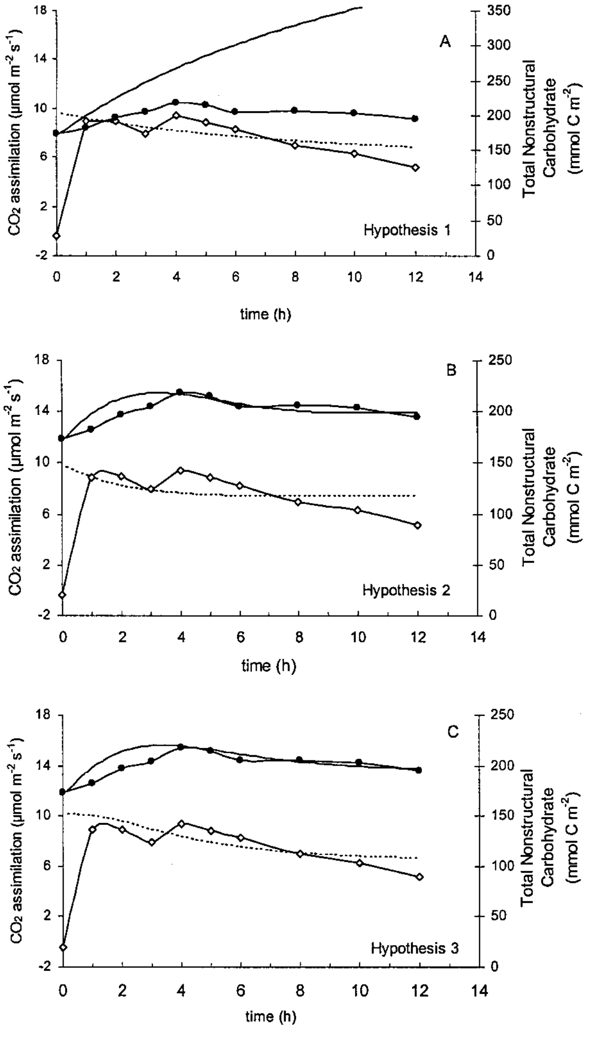

Fig. 5. Comparison of simulated (---) and observed (◇) carbon assimilation rates, and simulated (—) and observed (●) leaf carbohydrate content for the data shown in Fig. 2, for each of the three feedback hypotheses (Hyp. 1–3, A–C) shown in Fig. 4. Best-fit model parameters values for each hypothesis are given in Table 2.

made (Minchin *et al.*, 1993). For the most part, it was found that the addition of a dynamic feedback mechanism on assimilation rate did not alter in any qualitative way the partitioning behaviour found before (Minchin *et al.*, 1993). For example, under hypothesis 3, when the demand of sink 1 (vm_1) is increased with vm_2 held fixed, F_1 increases and s_1 decreases, while s_2 varies to maintain an almost constant flux F_2 into sink 2 (cf. Minchin *et al.*, 1993, their Fig. 5). Also, when the source activity in this model ($A_{\max}$) is decreased in a system with two inequivalent sinks of fixed demand, say, with $vm_1 < vm_2$, the decrease in F_1 is smaller than that of F_2, reflecting the greater degree of saturation of sink 1, so that F_1/F_2 increases as source activity declines (Fig. 7A, cf. Minchin *et al.*, 1993, their Fig. 9, in which s_0 was varied).

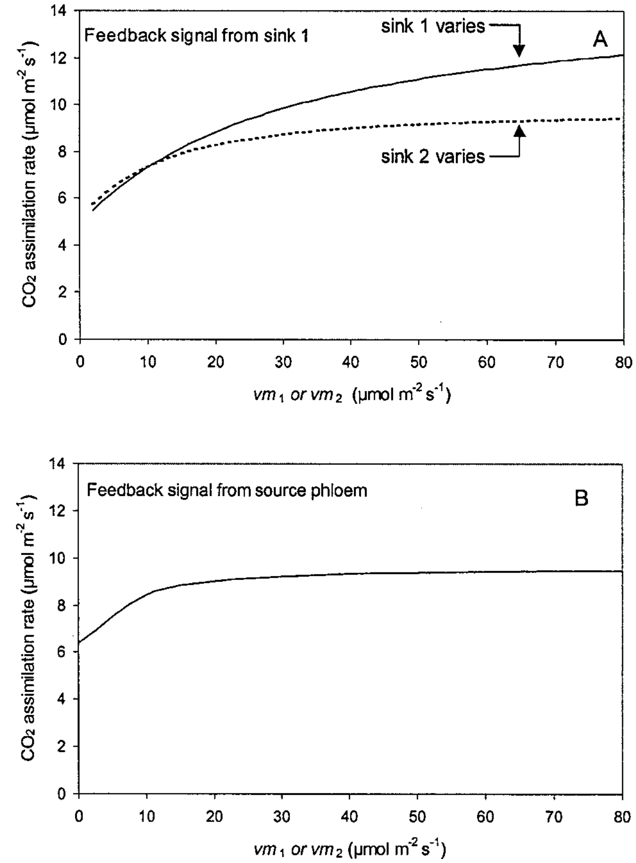

Fig. 6. Steady-state response of carbon assimilation to variation in sink demand predicted by the model. (A) Hypothesis 3 (feedback signal from sink 1 phloem), varying vm_1 with vm_2 fixed (—) and varying vm_2 with vm_1 fixed (---). (B) Hypothesis 2 (feedback signal from source phloem), responses to vm_1 and vm_2 are identical. The values of fixed parameters are given in Table 2.

With respect to partitioning behaviour, the only qualitative difference from the earlier model (Minchin *et al.*, 1993) lies in the predicted response of F_1 and F_2 to variation in the common pathway resistance α_0. Minchin *et al.* predicted a reduction in both F_1 and F_2 as α_0 is increased (Minchin *et al.*, 1993, their Fig. 7), because the source concentration s_0 in their model is fixed. By contrast, in this model s_0 is a dynamic variable. As a result there is little or no change in F_1 and F_2 as $\tilde{\alpha}_0$ varies (Fig. 7B). An increase in α_0 leads to a build-up of carbohydrate in the source phloem (s_0) which compensates the increase in α_0 such that the fluxes F_1 and F_2 and their total (F_0) are virtually unchanged in the steady state. Artificially fixing s_0 in the present model would lead to the behaviour predicted previously (Minchin *et al.*, 1993).

Discussion

The authors' main motivation for developing the present model has been the need for a simple representation

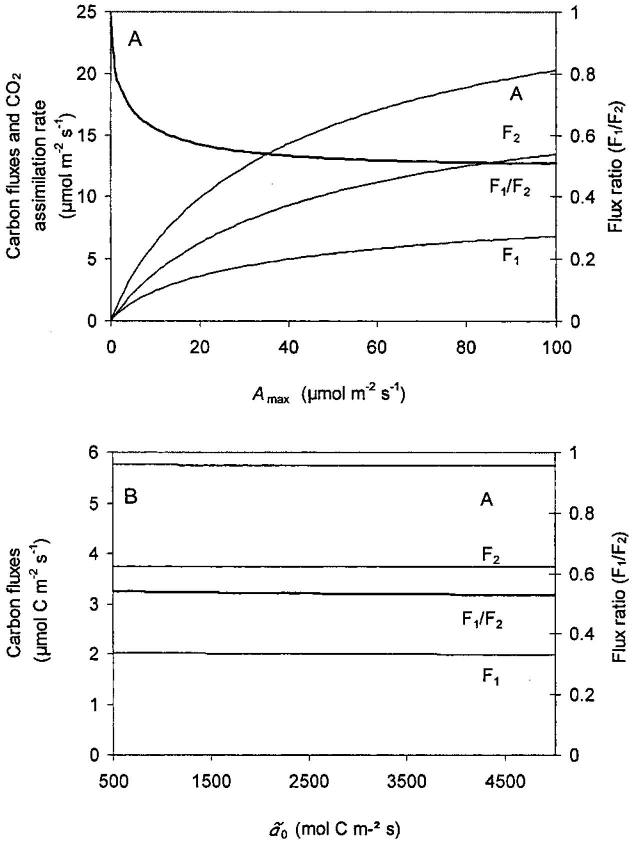

Fig. 7. Steady-state responses of carbon assimilation (A), assimilate fluxes to sinks 1 and 2 (F_1 and F_2), and their ratio F_1/F_2, to variation in (A), source capacity (A_{max}) and (B) the common pathway resistance ($\tilde{\sigma}_0$). The values of fixed parameters are given in Table 2.

of source–sink interactions for use in whole-plant models generally, and models of vine functioning in particular. In order to be of practical use in viticultural applications, and in view of current uncertainty regarding the underlying mechanisms, such a model should capture the essential features of source–sink interactions without attempting to represent the underlying biochemistry in great detail. The simple substrate-based source–sink transport models (Thornley, 1972; Dewar, 1993; Minchin *et al.*, 1993) therefore provide an appropriate starting point. In extending the model of Minchin *et al.* (Minchin *et al.*, 1993), nothing was assumed about the nature of the feedback mechanism other than that it is mediated by plant carbohydrate levels.

The experimental results support earlier findings (Correia *et al.*, 1990; Chaumont *et al.*, 1994), that leaf photosynthesis undergoes a diurnal decline during which total leaf carbohydrate content remains relatively constant. Correia *et al.* attributed the decline in photosynthesis to photoinhibition (Correia *et al.*, 1990), finding that both electron transport and carboxylation efficiency declined by 35% and 33%, respectively, between morning and afternoon under excessive light (1450 μmol m^{-2} s^{-1}). However, photoinhibition is unlikely to play a major role under normal environmental conditions (Chaumont *et al.*, 1994). Chaumont *et al.* found no evidence of photoinhibition during the mid-morning decline of photosynthesis, either in field-grown vines exposed to high PAR from morning under temperate or semi-arid climates, or in greenhouse vines under moderate temperatures (Chaumont *et al.*, 1994, 1997).

A modest diurnal decrease in the CO_2-saturated rate of photosynthesis was observed (11% between hours 2 and 10 of the photoperiod). This may or may not be associated with photoinhibition, although there was no significant change in the initial slope of the A/c_i curve. Because the operating point of the source leaf was confined to the CO_2-limited portion of the A/c_i curve, the observed decline in photosynthesis under ambient conditions occurred largely through stomatal limitation, with photoinhibition unlikely to have played a significant role. The fact that these results were obtained under controlled, non-limiting environmental conditions suggests that stomatal conductance is regulated by some internal factor other than plant water stress. A similar decline in photosynthesis in well-watered grapevines has been observed before (Correia *et al.*, 1990).

The sink–source feedback model is able to reproduce our experimental data, consistent with the view that the internal signal is linked to plant carbohydrate levels and thus to sink demand. These data and model simulations suggest that the feedback signal is unlikely to originate in the mesophyll (hypothesis 1), but may originate in the phloem of the source (hypothesis 2) or sinks (hypothesis 3). However, this conclusion is dependent on the mathematical form chosen to represent the negative feedback of carbohydrate levels on carbon assimilation (equation 5). It is possible that a different form, such as a threshold-delay response to mesophyll sucrose content, could reproduce the data. Hypothesis 1, therefore, cannot be excluded entirely, although the underlying mechanism for stomatal regulation by mesophyll sucrose content remains obscure.

Because only one leaf was illuminated in this experiment, one might have expected the plants to be source-limited rather than sink-limited. However, the following order-of-magnitude estimate of the sink–source activity ratio suggests otherwise. Daily assimilation by the source leaf was estimated at 4.3 mmol C d^{-1}, based on an average photosynthetic rate of 10 μmol C m^{-2} s^{-1} over a 12 h photoperiod and a leaf area of 100 cm^2. This level of source activity compares with an estimate of total sink demand of 4.2 mmol C d^{-1}, based on a total sink dry weight of 5 g with a 50% C content, a daily relative growth rate during the maturation phase of 1% for grapes (Ollat and Gaudillère, 1998) with a similar relative growth rate assumed for roots, and assuming that sink growth and maintenance respiration account for 50% of total sink demand. Therefore, source supply and sink demand are of the same order of magnitude, consistent with a feedback mechanism acting to maintain a balance between source and sink activities.

One might still question the reality of a sink feedback mechanism, on the basis that if such a mechanism were operating in plants with a single illuminated leaf, then in plants with many illuminated leaves the surplus of assimilates would lead to an unreasonably large down-regulation of photosynthesis. To address this concern, the daily cumulative PAR intercepted by whole plants under natural (greenhouse) light conditions was estimated. The plants used in the experiments had 7–10 leaves with a mean total leaf area of 720 cm^2. Using external PAR data collected at INRA Bordeaux during the 20 d prior to the experiment (January 2000, mean daylength = 9 h), and applying a measured greenhouse transmission fraction of 0.62, it was estimated that the whole plants intercepted an average of 0.38 mol PAR d^{-1} during this period. This is less than the 0.52 mol PAR d^{-1} intercepted by the single illuminated leaf (incident PAR = 1200 μmol m^{-2} s^{-1}, photoperiod = 12 h, leaf area = 100 cm^2). This comparison suggests that during the period concerned, the whole-plant source activity under natural light conditions was unlikely to have been greater than that for the single illuminated leaf, and therefore that the degree of down-regulation in whole plants would not have been unreasonably large.

Although other mechanisms for the observed diurnal decline in photosynthesis, such as photoinhibition, endogenous circadian rhythm, or local hydraulic signals in the leaf cannot be excluded entirely, carbon build-up in the sinks coupled with sink-to-source signalling appears to be the simplest mechanistic model able to describe these data.

It is suggested that, regardless of the underlying mechanism, a phloem-based feedback mechanism in the form of equation 5 provides a useful working hypothesis for incorporating source–sink interactions into plant models, which can be developed and tested further. The effectiveness of such a phloem-based feedback mechanism was illustrated by the predicted steady-state response of photosynthesis to sink demand (Fig. 6A, B). Under hypothesis 3, for example, the model predicts a positive feedback between sink demand and photosynthesis, regardless of the identity of the sink emitting the hypothetical signal, and of the sink whose demand varies. The model effectively describes a complete interdependence between all pools, i.e. between different sinks, as well as between sinks and source, mediated by sink-to-source signalling and by linkage through the phloem transport network.

Conclusion

Incorporation of a dynamic sink–source feedback mechanism into the model of Minchin *et al.* (Minchin *et al.*, 1993) introduces a positive dependence of carbon assimilation on sink demand, but does not alter the manner in which this carbon is partitioned between competing sinks. On the basis of the data from this study, other mechanisms for the observed diurnal decline in photosynthesis, such as photoinhibition, endogenous circadian rhythm or local hydraulic signals in the leaf cannot be excluded. Nevertheless, the phloem-based feedback model presented here is in reasonable agreement with observations, is open to further development and testing, and provides a useful working hypothesis for incorporation into plant growth models.

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Appendix 1 – Volume dependence

In equations (2a) and (2b) for the transport fluxes F_1 and F_2, the units of s_0, s_1 and s_2 are mol C m^{-3} phloem and the units of α_i are mol C m^{-6} s. Substituting $s_i = C_i/V_i$ (for i = 0, 1, 2) where C_i and V_i are, respectively, the relevant phloem carbohydrate pool (mol C) and volume (m^3), equations (2a) and (2b) can be written as:

$$F_1 = \frac{C_0\left[\tilde{\alpha}_2\left(C_0 - \frac{V_0}{V_1}C_1\right) + \tilde{\alpha}_0\left(\frac{V_0}{V_2}C_2 - \frac{V_0}{V_1}C_1\right)\right]}{\tilde{\alpha}_0(\tilde{\alpha}_1 + \tilde{\alpha}_2) + \tilde{\alpha}_1\tilde{\alpha}_2} \tag{A1}$$

$$F_2 = \frac{C_0\left[\tilde{\alpha}_1\left(C_0 - \frac{V_0}{V_2}C_2\right) + \tilde{\alpha}_0\left(\frac{V_0}{V_1}C_1 - \frac{V_0}{V_2}C_2\right)\right]}{\tilde{\alpha}_0(\tilde{\alpha}_1 + \tilde{\alpha}_2) + \tilde{\alpha}_1\tilde{\alpha}_2} \tag{A2}$$

where the $\tilde{\alpha}_i = \alpha_i \times V_0^2$ (i = 0, 1, 2) are rescaled transport resistances with units of mol C s. The rescaled parameters $\tilde{\alpha}_i$, rather than the α_i, were fitted to the experimental data. In the absence of direct estimates of the volume ratios V_0/V_1 and V_0/V_2, different values of V_0/V_1 and V_0/V_2 were considered over the range 0.1 to 1.0. Satisfactory model fits as in Fig. 5B, 5C were obtained in all cases (data not shown). The results shown in Figs 5–7 are for $V_0/V_1 = V_0/V_2 = 1$.

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