Modelling the Effects of Leaf-to-Fruit Ratio on Dry and Fresh Mass Accumulation in Ripening Grape Berries

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Abstract

Process-based fruit growth models are useful frameworks to advance our understanding of fruit quality build-up in response to climate and management techniques. In the present study, we adapted a biophysical model originally designed for peach (Fishman and Génard, 1998) to grapevines, to simulate the effects of various leaf-to-fruit ratios on fresh and dry mass accumulation in a ripening berry. The model represents a virtual mean berry during the post-veraison developmental stage, which is assumed to behave as a single cell separated with a composite membrane from the parent vine and the outside environment. Water accumulation was calculated through the water balance between xylem and phloem water influx and transpirational water loss, controlled by water potential gradient between the berry and the parent vine. Meanwhile, dry mass accumulation was simulated with the balance between phloem sugar import and respiration carbon depletion. The inputs of the model included initial fresh and dry mass, phloem sugar concentration, xylem water potential, fruit temperature and air humidity. In 2006, experimental data were acquired on *Vitis vinifera* cv. Cabernet Sauvignon fruiting cuttings grown in greenhouse and submitted to three leaf-to-fruit ratios at veraison (control, low and recovery). The dataset of the control treatment was used to calibrate the model, while the dataset of low and recovery treatments were used to validate the model. The predictions of the model were in agreement with the observed data. The reduced berry fresh and dry mass caused by the low leaf-to-fruit ratio and the recovery growth corresponding to the alternation leaf-to-fruit ratio from low to control level could be accurately simulated with corresponding phloem sugar concentration. Furthermore, the within cluster variation in berry fresh and dry mass under the same treatment were also well simulated when the different initial fresh and dry masses were considered. Simulations of diurnal changes in fresh mass and berry water balance were also demonstrated and discussed.

INTRODUCTION

Grapes are non-climacteric fruits that exhibit a double-sigmoid pattern of development, with the two distinct phases of growth separated by a lag phase called 'veraison' (Coombe, 1992; Robinson and Davies, 2000). As with most fleshy fruits, water and carbon flows into and out of berries are crucial for volumetric growth and accumulation of primary compounds, which determine the final fruit composition and quality (Coombe and McCarthy, 2000). These flows vary with fruit developmental stage and external conditions. Assimilate requirements to support dry matter accumulation and respiratory activities are different for the two periods of growth (Ollat et al., 2002). The flow rate of imported carbon during the first growth period is typically one-third of that required during the second growth period (Ollat and Gaudillère, 1996), partly due to a shift at veraison of phloem sugar unloading from the symplasmic to the apoplasmic pathway, that allows high levels of soluble hexoses to accumulate (Zhang et al., 2006). At

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the same time, the water budget of the berry shifts from a combination of xylem and phloem water supply to predominantly phloem (Greenspan et al., 1994; Bondada et al., 2005; Rogiers et al., 2006). This change is associated with an apparent uncoupling of fruit water status from plant water status, and may play a role in the well-known ability of grapes to continue to accumulate substantial amounts of solutes under limited soil water availability (Keller et al., 2006).

Environmental conditions, and some plant management techniques such as source-sink manipulations, play important roles in the control of water and solute transport and accumulation in the grape berry. These roles can be better understood through the use of a mechanistic model that describes the complex processes involved in fruit growth and analyses their relationship to the external conditions (Lescourret and Génard, 2005). Such a virtual fruit model could then provide an underlying framework to advance our understanding of fruit quality build-up (Génard et al., 2007) and would be an original approach for the grape.

In this paper, we present a model of berry growth, which is adopted from a peach model (Fishman and Génard, 1998), with adjustments to account for specific physiological traits of grape. Using the model, we evaluate the effects of various assimilate supply regimes on diurnal water balance and seasonal dry matter accumulation in a ripening berry by manipulating the leaf-to-fruit ratio. Comparisons of simulated and observed fruit growth were performed to test the model. The effect of initial fruit mass at the beginning of simulation on berry growth and final size were assessed.

MATERIALS AND METHODS

Plant Material and Leaf-to-Fruit Ratio Treatments

Fruiting cuttings of *Vitis vinifera* cv. Cabernet Sauvignon were prepared as described in Ollat et al. (1998). After pre-rooting, cuttings were transplanted into small pots (0.5 L) containing a mixture of perlite, sand and vermiculite (1:1:1) and transferred to an environment-controlled greenhouse in spring 2006. Full strength Hoagland's solution was supplied to each pot with a drip irrigation system 3–5 times per day to avoid any water stress throughout the experimental period. To simplify source-sink relationships, only one shoot and cluster were retained for each plant. The shoot was topped when the total leaf number reached 16. Lateral shoots were removed as soon as they appeared throughout the course of the experiment. Temperature and relative humidity in the greenhouse, which were needed as model inputs, were recorded hourly with two HOBO sensors (Onset Computer Corp., Bourne, MA, USA). During the experimental period, the hourly mean temperature and relative humidity varied from 13.4 to 41.9 °C, and 24.5% to 95%, respectively. Photosynthetically active radiation at plant height was measured by a Quantum Sensor (LI-COR, Lincoln, Nebraska, USA) and was estimated at about 60% of the outside conditions.

At veraison (50% clusters changed color), 110 uniformly growing plants were subjected to three leaf-to-fruit ratio treatments. Leaf removal was applied to obtain a leaf-to-fruit ratio of 9 leaves/70 berries for control plants (Control), and 4 leaves/70 berries for low treatment (Low). An alternation of these two ratios during experimental period were used for the third treatment (Recovery), in which plants were maintained at the low ratio ratio of 4 leaves/70 berries for the first 20 days, and thereafter some berries were removed to obtain a ratio of 4 leaves/30 berries which was similar to the control treatment. A randomized, complete block design was used with five replicates for each treatment. Grape berries were harvested 9 times at 5-day intervals. At each harvest, total fresh weight, pedicel fresh weight, and berry number per cluster were determined and mean berry fresh weight per cluster was calculated. To obtain the allometric relationship between fruit weight and surface area, the diameters of fifteen randomly chosen berries were measured with electronic digital calipers (Codiam Scientific, France). These same 15 berries were later dried in a forced-air oven at 70°C for at least 4 days and weighted. Berry water content was calculated as the difference between fresh and dry weight.

Model Description

The present model is an adaptation of a fruit growth model originally developed for peach and is described in details elsewhere (Fishman and Génard, 1998; Lescourret and Génard, 2005). Briefly, it is based on a biophysical representation of water and solutes transport, which is coupled with cell wall extension, stimulated by turgor pressure, and simulates growth of an individual grape berry at an hourly time step during the post-veraison developmental stage when there is no cell division. This assumes that (i) the berry is as one compartment (i.e., a cell community with a constant number of growing cells) separated with a composite membrane from the parent vine and the outside environment, and (ii) the Lockhart equation originally applied to a single cell describes the effect of hydrostatic pressure on the irreversible cell wall expansion in this mean compartment (Lockhart, 1965).

In the model, water enters the berry from xylem and phloem, but most of the post-veraison gain is due to water import from the phloem (Keller et al., 2006). The water flows from xylem or phloem into the fruit are described by equations based on differences in hydrostatic and osmotic pressures between xylem or phloem and the berry. Osmotic pressure is calculated from solute concentration. Fruit turgor potential is calculated by solving Lockhart's equation describing volume growth of the fruit. Water loss through berry transpiration is assumed to be proportional to the fruit surface area and to be driven by the difference in relative humidity between the air-filled space within the fruit and the ambient atmosphere. The transport of sugars from phloem to the fruit mesocarp is described by three possible mechanisms (as reviewed by Conde et al., 2007): (i) mass flow, which is proportional to the solution flow, (ii) passive diffusion, with the gradient of the sugar concentrations between phloem sap and berry flesh as driving force, (iii) active transport mechanism described by a modified Michaelis-Menten equation. Respiration, determined by berry growth rate and fruit temperature, leads to dry matter loss.

The hourly information coming from the external compartments includes temperature and relative humidity of the ambient atmosphere, water potential in xylem vessels and sugar concentration in phloem sap. The final outputs of the model are the diurnal and seasonal dynamics of both dry mass and water in the fleshy part of the berry. Initial conditions and inputs values for the model are summarized in Table 1.

Parameter Estimation and Model Calibration

The complete set of parameters used in the model is presented in Table 2. Parameters which had not been determined by independent experiments or taken from the literature were estimated through model calibration, by fitting the simulated outputs to the observations of the control treatment, using the nonlinear least squares method (R software, 'nls' function). The dataset of the control treatment was used to calibrate the model, while the dataset of low and recovery treatments were used to validate the model. To assess the goodness-of-fit and the predictive quality of the model, two commonly used criteria, root mean squared error (RMSE) and relative root mean squared error (RRMSE), were adopted (Kobayashi and Us Salam, 2000). RMSE is given as:

$$RMSE = \sqrt{\frac{1}{N}\sum n_i (S_i - \overline{M}_i)^2}$$

where, n_i, S_i and $\overline{M}_i$ are the number of measured values, the simulated values and the mean of measured values for the i-th date, respectively. N is the total number of measured data. The RRMSE is calculated as:

$$RRMSE = \frac{RMSE}{\overline{M}}$$

where, $\overline{M}$ is the mean of all measured values.

The lower the value of RMSE and RRMSE, the better the goodness of fit and predictive quality. Usually, RRMSE values lower than 0.25 are considered as suitable but values higher than 0.5 are unacceptable.

RESULTS AND DISCUSSION

The model was able to reproduce the pattern of post-veraison berry growth as well as the marked effect of leaf-to-fruit ratio on final fresh and dry mass, even on data not used for model calibration (Fig. 1). The RRMSE of dataset used for model calibration, indicating the goodness of fit, was 0.071 for berry fresh growth and 0.11 for berry dry mass growth, which are both satisfactory (Fig. 1A, D). The predictive quality of the model was evaluated against independent data under various leaf-to-fruit ratio treatments (Low and Recovery) (Fig. 1B, C, E, F). The predictive quality criteria (RRMSE) were 0.061 and 0.064 for fresh and dry mass growth, respectively, indicating that the model correctly simulated the negative effect of low leaf-to-fruit ratio on berry growth. More interestingly, the model performed well in simulating the recovery of berry fresh and dry mass increase due to the increase of leaf-to-fruit ratio from low to control level (RMSE 0.074 and 0.081, respectively).

In the model, the leaf-to-fruit ratio effect was simulated by the mass concentration of sugar in the phloem. For control treatment, the minimum and maximum mass concentrations were 0.17 and 0.25; whereas for the limiting C supply (i.e., low leaf-to-fruit ratio) treatment, lower sugar concentrations were used 0.12 and 0.20, respectively. The reliability of these estimates needs to be validated experimentally. This is particularly true for their diurnal and seasonal dynamics. To date, such direct measurements on diurnal phloem sugar concentration are scarce in the literature, probably because physiological studies on functional phloem are notoriously difficult.

The berry fresh weight at veraison varied within a cluster (Fig. 2A) (Tarter and Keuter, 2005). Three major subclasses of berries, accounting 91.7% of the total berries in a cluster, were used to evaluate the model to simulate variation within the cluster. For the experimental data, when the initial berry weight was high, the cumulative berry growth quickly reached its maximum at 0.27 g dry wt. In contrast, when initial weight was low, the growth rate was increased but the fruit never grew very large because solute influx was limited. Simulations performed with different initial fruit weights emphasized the importance of the initial value in determining the pattern of the growth curve and fruit weight at harvest. When different initial fresh and dry weights were taken into account, the model adequately simulated the different growth dynamics of the three subclasses (Fig. 2B, C), except for the berry having the lowest initial fruit mass. The RRMSE was 0.038 and 0.052 for berry fresh and dry mass growth, respectively. These values were smaller than those obtained when the initial fresh and dry weight used the values of the mean of all berries in a cluster (Fig. 1), indicating the initial fresh and dry weight modified the predictive quality of the model. These model characteristics indicate the possibility to simulate berry-to-berry variation within one cluster. The potential of the present model to simulate the within cluster variation will be further studied.

Simulations of diurnal dynamics of berry fresh weight and water balance were illustrated for a 4-day post-veraison period under conditions denoted in Table 1 as control (Fig. 3). The model correctly reproduced the diurnal pattern of berry expansion and contraction commonly observed in ripening grapes (Matthews and Shackel, 2005). Simulated berry growth occurred during the night time when net water flow into the berry exceeded net water loss through transpiration. In contrast, day periods were characterized by berry contraction where the magnitude depended mainly on the transpiration rate. In the model, variations in daily water flow into the berry were controlled by the water potentials of the fruit and the plant. Theoretically, berry transpiration has the potential to decrease berry water potential: transpirational water loss increases the sugar concentration and consequently decrease the berry osmotic potential. Thus, the demand for water and soluble matter by the fruit was partly generated by fruit transpiration. Rogiers et al. (2001) reported that berry transpiration is highly dependent on VPD and usually declines during ripening, which implies that evaporative water loss ceases to function as the driving force for water influx at maturity (Dreier et al., 2000). Our model is partially consistent with this observation, as the skin water permeability parameter decreased over time, reducing the transpiration rate on both a whole-berry and surface area basis. The

simulation of diurnal dynamics of berry growth will continue to be validated against the observed dataset.

In conclusion, the model is able to describe the growth of an individual berry on a diurnal and a seasonal basis taking into consideration only fundamental biophysical processes and their response to external conditions. Indeed it accurately predicted mean berry dry mass in response to different source-sink levels (initial fruit dry mass and leaf-to-fruit ratio). Additional physiological characteristics of the grape berry might be included in future development of the model: e.g. xylem backflow. This is a process by which surplus water is withdrawn from the berry and recirculated to other parts of the plant (Lang and Thorpe, 1989; Keller et al., 2006), and it might play a role in the berry water budget.

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Tables

Table 1. Values of input variables and initial conditions used to generate berry growth simulations presented in Figures 1 and 2. The notation S_0, W_0, Cp_{min}, Cp_{max}, Ψ_{min} and Ψ_{max} represent initial dry weight (g), initial fresh weight (g), daily minimum and maximum phloem sugar concentrations (g g^{-1}), and daily minimum and maximum xylem water potentials (MPa), respectively. Symbols □, ◊, and ∇ correspond to the berry subclasses defined in Figure 2.

	Run 1. Effect of leaf-to-fruit ratio				Run 2. Variation within a cluster		
	Control	Low	Recovery		Berry subclasses		
			Low	Control	□	◊	∇
S_0	0.088	0.088	0.088	-	0.099	0.15	0.20
W_0	0.55	0.55	0.55	-	0.5	0.7	0.9
Cp_{min}	0.17	0.12	0.12	0.37	0.17	0.17	0.17
Cp_{max}	0.25	0.20	0.20	0.45	0.25	0.25	0.25
Ψ_{min}	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0
Ψ_{max}	-0.1	-0.1	-0.1	-0.1	-0.1	-0.1	-0.1

Table 2. List of parameters and their values for the grape berry growth model.

Parameters	Unit	Value	Definition	Source
Respiration				
qg	g C g^{-1} dw	0.019	Growth respiration coefficient	Lit.
qm	g C g^{-1} dw	0.59×10^{-4}	Maintenance respiration coefficient	Lit.
Q_{10}	dimensionless	1.65	Temperature ratio of maintenance respiration	Lit.
Transpiration				
ρ_1, ρ_2, ρ_3	dimensionless	107, -15.4, 54.9	Parameters related to skin water permeability	Exp.
β, γ	dimensionless	4.15 , 0.71	Parameters for calculating berry surface area	Exp.
Cell growth				
ϕ	bar^{-1} h^{-1}	0.01	Cell wall extensibility coefficient	Lit.
Y	bar	3.0	Threshold value of turgor pressure for growth	Lit.
Water and sugar transport				
V_{max}	g sucrose g^{-1} dw h^{-1}	0.0031	Maximum rate of active sugar uptake	Lit.
K_m	dimensionless	0.08	Michaelis constant for active sugar uptake equation	Lit.
τ	h	160	Inflexion time controlling active sugar uptake	Cal.
δ	h	270	Kinetic parameter for sugar active uptake	Cal.
P_s	g cm^{-2} h^{-1}	0.0027	Solute permeability of composite membrane	Lit.
a_p	dimensionless	0.0035	Ratio between area of composite membrane and berry surface	Lit.
σ	dimensionless	0.9	Reflection coefficient of composite membrane for sugar	Lit.
L_{p1}, L_{p2} L_{p3}, L_{p4}	dimensionless	0.006, 0.0015, 250, 0.009	Parameters related to water conductivity of the composite membrane	Cal.

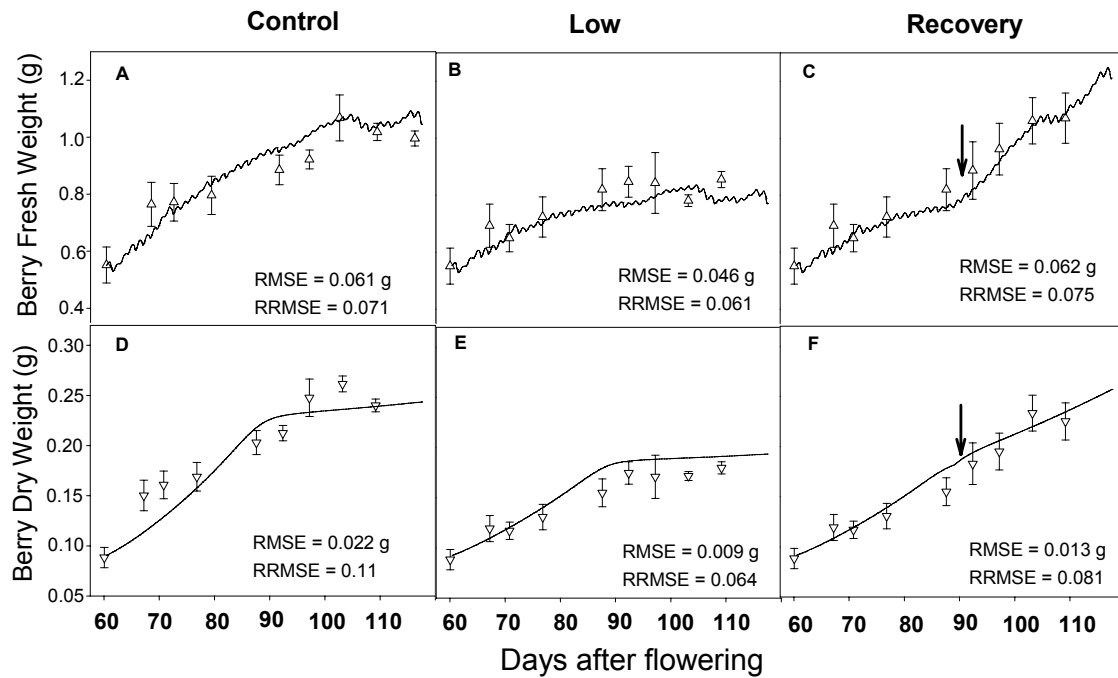Cal.=Calibration, Exp.=Experiment, Lit.=Literature

Figures

Fig. 1. Measured (symbols) and simulated (lines) post-veraison dynamics of berry fresh (A, B, C) and dry weight (D, E, F) under three leaf-to-fruit ratio treatments (Control, Low, and Recovery). Arrows indicate the time shifting the leaf-to-fruit ratio from low to control level. Vertical bars represent standard deviations of measurements (n=5). The root mean squared error (RMSE) and relative root mean squared error (RRMSE) are indicated in each graph.
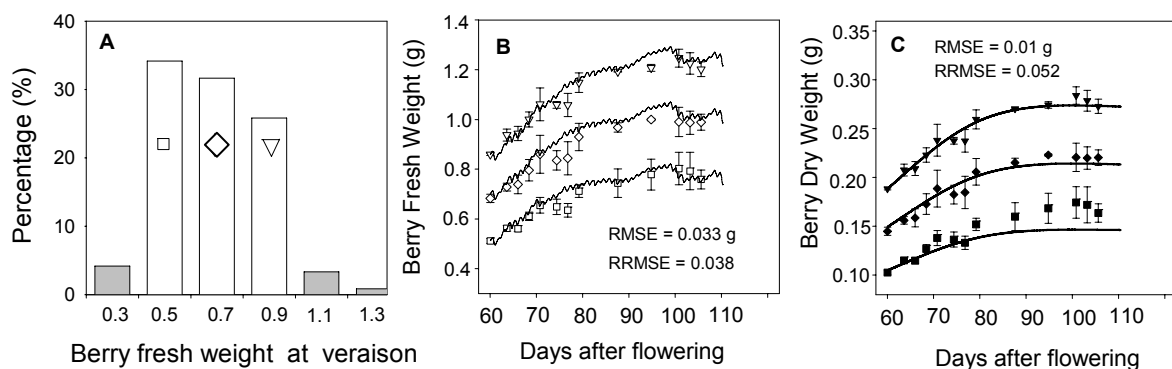

Fig. 2. Berry fresh weight distribution at veraison (A), measured (symbols) and simulated (lines) variation of berry fresh (B) and dry (C) weight within cluster of control treatment. Symbols □, ◊, and ∇ indicate three berry subclasses with an initial fresh weight of 0.5, 0.7, and 0.9 g, respectively. Vertical bars represent standard deviations of measurements (n=5). The root mean squared error (RMSE) and relative root mean squared error (RRMSE) are indicated in the graph.
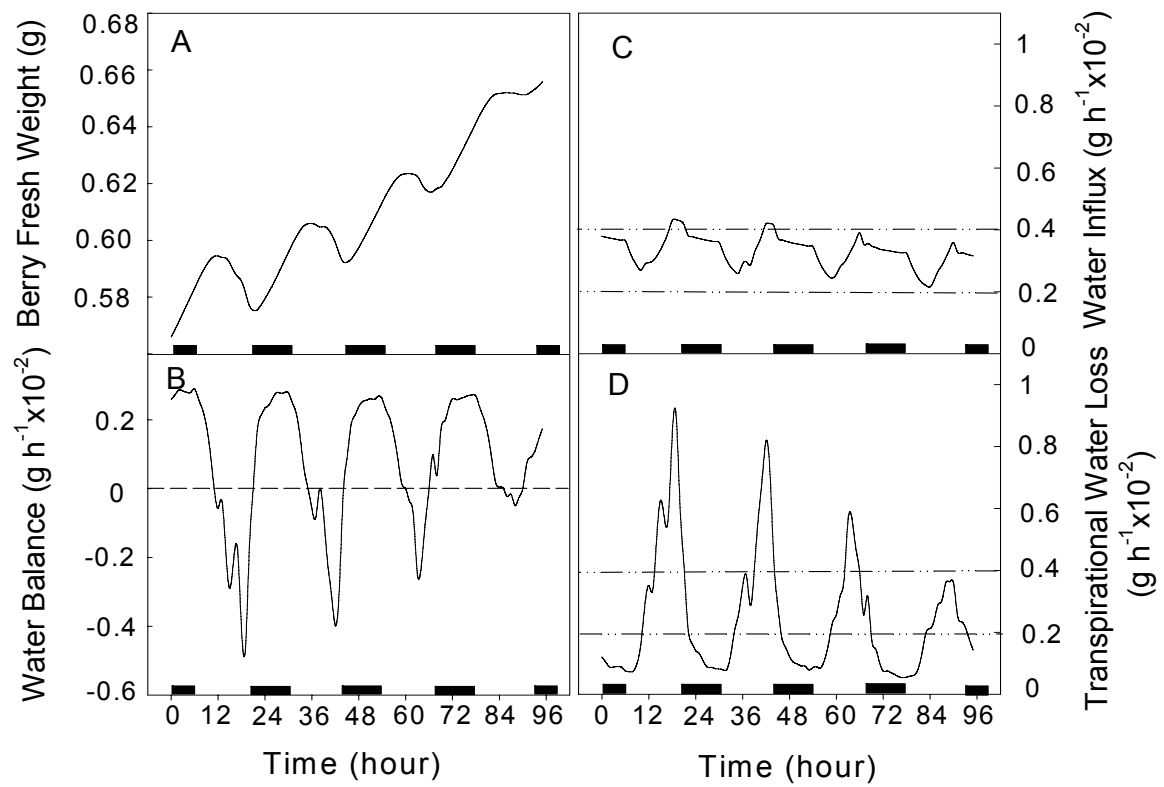
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Fig. 3. Simulations of diurnal dynamics of berry fresh weight (A), water balance (B), water influx (C), and transpirational water loss (D) within a 4-day period (63–66 days after flowering). To make the comparison clearer, common scales were used for (C) and (D). Black horizontal bars indicate the dark period.