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ARTICLE

# Analysis of blueberry (*Vaccinium corymbosum* L.) fruit water dynamics during growth using an ecophysiological model

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**ABSTRACT**

Blueberry (*Vaccinium corymbosum* L.) fruits exhibit three growth stages associated with distinct biological processes. During these periods, water and carbon accumulate in the fruit, determining quality traits such as fruit size and sugar concentration. We adapted the Fishman-Génard model to blueberry and used it to analyse the effect of fruit load on fruit fresh mass and water dynamics, based on empirical observations of dry mass and sugar content performed throughout the fruit growth period. Different fruit load treatments were imposed during two seasons on 'Brigitta' blueberry plants growing under different culture systems. Increasing fruit load significantly reduced the fresh mass of the fruits at harvest, but did not affect sugar concentration, which was simulated and validated with a mean error of 7% for fresh mass and 15% for sugar concentration for the tested conditions. The most sensitive model parameters were those related to cell wall extensibility and sugar uptake. The simulations indicated that larger fresh mass of the fruit was mainly caused by increases in water fluxes rather than pressure differences. The model implementation provides the first estimates of a set of parameters which govern blueberry fruit water dynamics from fruit set to harvest.

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**KEYWORDS**
Fruit load; sumulation; water relations

## Introduction

Blueberry fruit is classified as a true berry and exhibits three stages of growth (e.g. Coombe, 1976; Darnell, Stutte, Martin, Lang, Early, 1992; Godoy, Monterubbianesi, & Tognetti, 2008). These stages have been associated with distinct biological processes: in stage I, a rapid increase of pericarp size follows fertilisation due to increasing cell number; in stage II, a retarded development of the pericarp is coincident with a rapid development of the embryo and endosperm tissues; in stage III, a second rapid pericarp development takes place due to cell expansion until fruit ripening (Darnell et al., 1992). During these development periods, the water and carbon balance (i.e. the result of incoming and outgoing fluxes of water and carbon) determines the rates of matter accumulation in the fruit, thereby determining the final fruit size, water content, and content of carbon compounds such as sugars. These variables are the main criteria for assessing the quality of fresh fruits, which has become an increasingly important aspect of fruit production (Grechi, Hilgerts, Génard, & Lescourret, 2008) and consumer expectations (Albornoz, Ortega, Segovia, Bracho, & Cubillan, 2009; Jaeger & MacFie, 2001).

It is known that blueberry fruit size can be controlled by adjusting the fruit load via pruning (e.g. Seifker & Hancock, 1987, Strik, Buller, & Hellman, 2003). Such adjustment, which regulates the photoassimilate availability for the competing fruits, determines the magnitude of structural component synthesis and solute accumulation in the fruit. The changes in solute concentration create a gradient between the water potentials in the stem and in the fruit, which leads to water uptake by the fruit through the season. A larger fruit weight in peach (Morandi, Grappadelli, Rieger, & Lo Bianco, 2008) and apple (Stopar, Bolcina, Vanzo, & Vrhovsek, 2002), is obtained at low fruit loads and is closely related to higher fruit sugar content, promoting cell size. However, as opposed to fruit weight, sugar content had a small variation in response to fruit load in tomato genotypes (Prudent et al., 2009). In blueberry, developmental increase in fresh weight is parallel to that of soluble sugars (Darnell, Cano-Medrano, Koch, & Avery, 1994). A greater weight and sugar content in blueberry fruit, induced by decreasing flower bud density in blueberry shoots (Maust, Williamson, & Darnell, 1999), suggest the significance of the osmotic effect on fruit growth. However, it seems

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that cell number, not cell size, may be the primary determinant of final blueberry fruit size (Johnson, Malladi, & NeSmith, 2011; Swain & Darnell, 2002).

Although blueberry fruit growth and quality have been studied through the manipulation of fruit load and pruning (e.g. Jorquera-Fontena, Alberdi, & Franck, 2014; Maust et al., 1999; Strik et al., 2003), the effect of these practices on variables related to water and sugars interacting throughout fruit development has not been studied up to now. Because water and sugar accumulation in the fruit is a result of several linked processes, the development of ecophysiological models has been proposed as the best tool for understanding these processes (Génard & Lescourret, 2004; Sadras, Collins, & Soar, 2008). Unlike for other fruit trees, ecophysiological models have not been developed for or adapted to blueberry fruit, which raises an opportunity to increase the knowledge about this species which is especially relevant considering the increasing market demand for this fruit due to its benefits to human health.

Our working hypothesis was that the effect of fruit load on blueberry fresh fruit mass is explained not only by changes in fruit carbon content but also by differences in fruit water dynamics brought about by altered soluble sugar dynamics. The aim of this study was to adapt the model proposed for modeling peach fruit growth by Fishman and Génard (1998) to blueberry fruit in order to use it for analysing and simulating seasonal variations of fresh fruit mass, water content, and sugar concentration in response to differences in fruit sugar dynamics brought about by a range of fruit load treatments. The model is a biophysical representation of water accumulating in the fruit, which is the main component of fresh mass in blueberry fruit (83–90%; Adams 1975; Kalt et al., 2003). We here present and discuss the first estimated values of several parameters driving blueberry fruit growth and water dynamics.

## Materials and methods

### Model description and features

The model is based on a biophysical representation of fruit growth, originally adjusted for predicting the period of rapid fruit growth of peach (Fishman & Génard, 1998). The fruit is described as a single homogeneous compartment, separated from the ambient environment by a composite membrane, where the main variable of the system is the fruit water mass ($w$) depending on fluxes and water relations (water influx, fruit transpiration, and osmotic and turgor pressures). Fruit dry mass ($M_d$) and sugar content at different fruit growth stages were here considered as model inputs, which allowed us: (1) to add all phases of blueberry fruit growth since $M_d$ accounts both for the structural matter gain due to cell division and for the synthesis and incorporation of new cell wall and its components; (2) to calculate the dynamic of transformation of carbon (C) into sugar throughout the growth period for simulating sugar concentration and its effect on osmotic potential. The model interacts with two environmental variables, daily air temperature and humidity, entering as model inputs. The processes involved are interrelated by feedback loops which act as an internal control of the system (Fishman & Génard, 1998).

### Water fluxes

The rate of change in the amount of water in the fruit with time ($dw/dt$, g $d^{-1}$) is the algebraic sum of the water inflow from xylem and phloem ($U$, g·$d^{-1}$) and the water outflow due to fruit transpiration ($T_f$, g·$d^{-1}$):

$$dw/dt = U - T_f \tag{1}$$

The $T_f$, leading to mass loss, is assumed to be proportional to the fruit surface area ($A_f$), and to be driven by the difference in relative humidity between the air-filled space within the fruit ($H_f$, assumed to be equal to 100%) and the atmosphere ($H_a$):

$$T_f = A_f \alpha \rho (H_f - H_a) \tag{2}$$

where $A_f$ is fruit surface area (cm$^2$), $\rho$ is the fruit surface conductance (cm·h$^{-1}$, permeation coefficient of the fruit surface to water vapour), and $\alpha = M_W P^*/RT$, with $M_W = 18$ g·mol$^{-1}$ being the molecular mass of water, $P^*$ the saturation vapor pressure according to the description of Fishman and Génard (1998), $T$ temperature in Kelvin ($K$), and $R$ the gas constant (83 cm$^3$·bar·mol$^{-1}$·K$^{-1}$). The $A_f$ was linked to fruit fresh mass ($M_f$) through an allometric relationship, in which the empirical parameters ($y$ and $z$) depend on fruit geometry:

$$A_f = y(M_f)^z \tag{3}$$

In the model, the xylem and the phloem were assumed to be separated from the fruit cells by a membrane which was considered to be fully impermeable to sugars and solutes (Nobel, 1974). This allowed defining the water influxes into the fruit ($U$, g·d$^{-1}$) as follows:

$$U = A_f a L(\psi_s - \psi_f) \tag{4}$$

where a dimensionless constant of proportionality ($a$) represents the area of the vascular network per fruit surface area, $L$ is the xylem and phloem hydraulic conductivity (g·cm$^{-2}$·bar$^{-1}$·d$^{-1}$) between stem and fruit, and $\psi$ (bar) is the water potential of stem and fruit (subscript $s$ and $f$, respectively).

### Fruit water potential

The fruit water potential ($\psi_f$) was equal to the difference of turgor ($P_f$, bar) and osmotic pressures ($\pi_f$, bar). The $\pi_f$ is given by:

$$\pi_f = \frac{RT\sum^m}{w} \tag{5}$$

where $T$ is temperature in Kelvin (K), $R$ is the gas constant (83 $\text{cm}^3\cdot\text{bar}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$), $w$ is fruit water content ($\text{cm}^3$), and $m$ is the number of moles of osmotically active solutes. The $m$ was calculated by division of the mass of the present solutes (g) by their corresponding molecular masses ($\text{g}\cdot\text{mol}^{-1}$). As a first modeling approach, $m$ was calculated as the sum of osmotically active solutes obtained from soluble sugars and potassium content, due to their greater importance in osmotic pressure (Sharp, Hsiao, & Silk, 1990). Because other compounds such as organic acids and amino acids are also relatively important in osmotic pressure, an additional osmotic pressure (parameter $K_5$, bar) was calculated via model calibration (see section 'Model parameterisation') to then be summed to the osmotic pressure given by sugars and potassium. Potassium (g) was considered as a fixed mass proportion of fruit dry mass accumulated ($M_d$), while soluble sugar content was calculated by integrating Equation (6) proposed by Grechi et al. (2008), which is based on a balance of C flow in the fruit:

$$\frac{dCTS(t)}{dt} = CC_{fruit}\frac{dM_d(t)}{dt} - kCTS(t) \tag{6}$$

where $dCTS/dt$ is the rate of total soluble sugar influx ($\text{gC}\cdot\text{d}^{-1}$), $CC_{fruit}$ is the carbon concentration in the fruit dry mass (gC·g$M_d$–1), $dM_d/dt$ is the growth rate of the dry mass ($\text{g}\cdot\text{d}^{-1}$), $CTS$ is the carbon content from the sum of different kinds of sugars in the fruit (gC), and $k$ is the relative rate of transformation of carbon present in sugars into compounds other than sugars ($\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$). The integrated Equation (6) gives a result in carbon units, which must then be divided by the carbon content of each sugar found in the fruit in order to satisfy Equation (5). This requires to define the seasonal dynamics of each sugar due to their different molecular masses and hence osmotic pressures. However, as only hexoses were found in the present study (see section 'Model parameterisation'), it was not necessary to define the above-mentioned dynamics and, thus, a global pool of sugars (g) was considered. We determined (see section 'Model parameterisation') that $k$ mainly depends both on time elapsed since fruit set (in days after bloom, $DAB$) and fruit relative growth rate ($RGR$):

$$k = k_1(RGR)^{k_2}\exp^{(-k_3DAB)} \tag{7}$$

where $K_1$, $K_2$ and $K_3$ are empirical parameters.

Turgor pressure ($P_f$, bar) was calculated by solving Lockhart's equation (1965), which assumes that cell expansion occurs by an irreversible increase in cell volume ($V$, $\text{cm}^3$ that involves water uptake rate [$dV/dt$]), cell wall extensibility ($\phi$, bar $\text{d}^{-1}$) and a threshold value ($Y$, bar) of the fruit turgor pressure above which irreversible expansion occurs:

$$dV/dt = V\phi(P_f - Y) \tag{8}$$

The volume increase is the result of the increase of water and $M_d$ volume. However, as $M_d$ volume is much less than water volume, it can be neglected (Fishman & Génard, 1998). Since the $\phi$ value tends towards zero as fruit development becomes completed (Proseus, Ortega, & Boyer, 1999) we used the approach of Liu, Génard, Guichard, and Bertin (2007):

$$\phi = \frac{\phi\max}{1 + \exp^{(k_4DAB)}} \tag{9}$$

where $\phi$ max is the maximum wall extensibility and $K_4$ is an empirical parameter defining the seasonal changes of $\phi$. The decrease in $\phi$, resulting from Equation (9), is consistent with ontogenetic changes in the activities of pectin methyl esterases and poligalacturonases in the cell wall of blueberry fruit (Proctor & Miesle, 1991), which influence both growth and porosity properties of the cell wall (Bosch & Hepler, 2005).

Assuming water density at 1 g $\text{cm}^{-3}$ ($V \approx w$) and combining Equations (1), (2), (4), and (8), fruit turgor pressure was calculated as:

$$P_f = \left[aA_fL(\psi_s + \pi_f) - T_f + V\phi Y\right]/(aA_fL + V\phi), \left(\text{If } P_f \geq Y\right) \tag{10}$$

If $0 \leq P_f < Y$, $dV/dt = 0$, in this case Lockhart's equation is no longer valid, and $P_f$ was defined as:

$$P_f = \left[aA_fL(\psi_s + \pi_f) - T_f\right]/(aA_fL), \left(\text{if } 0 \leq P_f < Y\right) \tag{11}$$

After calculating the water fluxes, the fresh mass of the fruit was calculated integrating Equations (1), (2), and (4), plus fruit dry mass ($M_d$) over time ($t$):

$$M_f(t) = w_0 + \int(U - T_f)dt + M_d(t) \tag{12}$$

where $w_0$ is the initial water mass in the fruit.

Over the growth period, the total sugar concentration of the fruit ($SC$, $\text{g}\cdot\text{g}^{-1}$ $M_d$) was calculated by dividing the results obtained by integrating Equation (6) and fruit dry mass ($M_d$):

$$SC(t) = \frac{CTS(t)}{CC_{su}}\cdot\frac{1}{M_d(t)} \tag{13}$$

where $CTS(t)$ is accumulated sugars (g C) and $CC_{su}$ is C content of the sugar (as mentioned, C content in the hexose).

Finally, the model framework allows simulating other intermediate variables of the system, such as osmotic and turgor pressure and water influx and transpiration.

### *Plant material and experimental sites*

During the winters of 2009 and 2010, different reproductive bud-per-plant adjustments were established on middle-maturing cv. 'Brigitta' highbush blueberries (*Vaccinium corymbosum*) planted in 2004, growing in two commercial orchards located in the Region de La Araucanía in Chile (38°29′S, 72°23′W, site 1; and 38°58′S, 72°47′W, site 2) on an Andisol. The sites were managed under conventional (site 1) and organic (site 2) culture systems. Both sites belong to a temperate climate, with few differences between them. In general, the rainfall was distributed throughout the year, with the highest precipitation during winter and a moderate dry season of less than 4 months during summer. The thermal regime registered a mean of 8°C in the coldest month and 15°C in the warmest month. In each orchard, fertilisation, irrigation, and control of pests and diseases were applied according to the locally recommended practices.

### *Fruit measurement and treatments*

Data of fruit growth were collected from the plots during two consecutive productive seasons in site 1 (2009–2010 and 2010–2011) and during one productive season in site 2 (2010–2011). Measurements of fruit equatorial diameter were periodically performed with a digital calliper on plants belonging to one of four reproductive bud load treatments: low (L), medium (M), medium low (ML), and high (H) (Table 1). The treatments were obtained from un-pruned plants: (1) by slight speed-pruning criteria (H), (2) by conventional pruning criteria (M), and (3) by a severe conventional pruning (ML), plus (4) an additional manual bud removal (L). The ratios of reproductive bud loads relative to the H treatment corresponded to 0.5, 0.3, and 0.2 for M, ML, and L treatments, respectively. The pruning criteria were applied in accordance with Strik et al. (2003). For each treatment (for each season and site combination), three blocks of six plants were randomly placed in three orchard rows. In three plants of each block, two representative full sun-exposed clusters of fruits were selected, and five fruits per cluster were tagged for measuring their equatorial diameter ($di$) from fruit set until the moment when berries reached full blue colour development. The remaining plants, other than those used for diameter measurements, were used for determining fruit sugar concentration (see section 'Soluble sugar extraction and analysis'). The measured diameters were then used to estimate fruit fresh ($M_f$) and dry ($M_d$) masses by applying allometric functions ($M_f = 7.6 \cdot 10^{-4} di^{2.797}$, $n = 432$, $r^2 = 0.98$ and $M_d = 5.2 \cdot 10^{-5} di^{3.041}$, $n = 264$, $r^2 = 0.97$), which were previously developed and validated for the tested conditions (data not shown). The obtained results for each season and site combination were used either for estimating model parameters or for its local validation (Table 1). Fruit dry mass was obtained by drying at 70° C to a constant weight, and the initial mass of water (value used for integrating Equation 12) was estimated per treatment from the difference between fresh and dry masses of 30 fruits at the beginning of the measurement period.

### *Soluble sugar extraction and analysis*

Sun-exposed fruit clusters from the remaining three plants per block (other than those used for diameter measurements) were tagged to periodically collect fruit (average 14 days) and to determine its soluble sugar concentration through the season. Picked fruits were frozen to −80°C and then freeze dried. Sugars from ground freeze-dried fruits (50 mg) were extracted in 80% ethanol containing maltose (3 $g{\cdot}L^{-1}$) as internal standard, for 1 h at 60°C, and in distilled water under the same conditions. Each extraction was followed by a centrifugation at 13,000 × *g*. The pooled supernatants were used for sugar analysis. Soluble sugar extracts were analysed through high-performance thin-layer chromatography (HPTLC, CAMAG, Muttenz, Switzerland), in order to determine the sugar composition of fruits.

**Table 1.** Fruit load treatments applied to blueberry cv. 'Brigitta' growing in different systems of culture and seasons.

| | Number of reproductive buds per plant | | |
|---|---|---|---|
| | Site 1 (S1): conventional culture | | Site 2 (S2): organic culture |
| Fruit load treatment | 2009–2010 season | 2010–2011 season | 2010–2011 season |
| High (H) | 850.81 (± 21.05) | **695.96 (± 17.22)** | **600.11 (± 18.72)** |
| Medium (M) | **442.21 (± 24.07)** | **332.55 (± 16.87)** | 290.70 (± 14.89) |
| Medium low (ML) | 249.12 (± 17.39) | | |
| Low (L) | **183.97 (± 15.61)** | 140.01 (± 15.01) | **128.31 (± 13.34)** |

Mean values (± standard deviation) of reproductive buds, and abbreviations of treatments and sites, are presented. Bold letters indicate treatment used for model calibration and its internal validation. Treatments not in bold were used for external validation.

## Model inputs

The inputs of the main model were (1) fruit dry mass growth curve, (2) mean daily air temperature, and (3) daily mean air humidity. In addition to these variables, fruit soluble sugar content measured at different growth stages (described above) was also an input of the submodel used for estimating the relative rate of sugar transformation. The estimated fruit dry mass for each season and site combination was fitted using a logistic function with five parameters:

$$M_d(t) = \frac{A}{1 + e^{-(b + cDAB + dDAB^2 + eDAB^3)}} \tag{14}$$

where $A$ is the maximal dry mass (g), $DAB$ is time in days after bloom and $b$, $c$, $d$, and $e$ are empirical parameters defining the shape of the curve. This function has been used for other fruits with a double-sigmoid growth pattern (Opara, 2000). The adjusted growth curves exhibited a strong relationship between dry mass and days after bloom with $r^2$ ranging between 0.96 and 0.98 and $p$ values for coefficients ranging from 0.00117 and $2 \cdot 10^{-16}$ (data not shown).

Daily means of air temperature and relative humidity were collected from meteorological stations (Adcon Telemetry, Klosterneuburg, Austria) located near each orchard (data not shown).

## Model parameterisation

For estimating model parameters, we used the conditions highlighted in bold characters in Table 1.

The parameters obtained from independent measurements were: (1) the permeation coefficient ($\rho$, Equation 2), which was calculated as proposed by Gibert, Lescourret, Génard, Vercambre, and Pérez Pastor (2005) using 12 untagged fruits per treatment collected in the 2010–2011 season at different developmental stages (data not shown); and (2) the coefficients $y$ and $z$ of the allometric equation for estimating fruit surface area from fresh mass (Equation 3). Fruit surface area was estimated from the polar and equatorial diameters of fruit collected at different growth stages, assuming an ellipsoidal fruit geometry ($r^2$ = 96%, $n$ = 72; data not shown).

The parameters estimated via model calibration were: (1) conductivity of the composite membrane for water transport ($L$, Equation 4), (2) threshold value of turgor pressure ($Y$, Equation 8), (3) empirical parameters of cell wall extensibility ($\phi\max$ and $K_4$ ; Equation 9), and (4) pool of osmotically active solutes, other than sugars and potassium ($K_5$). For calibrating the parameters, a non-linear least-squares procedure was used to adjust simulated fruit fresh mass to observed fresh mass. Due to the high number of parameters solved via calibration a stepwise adjustment was performed. Empirical parameters $K_4$ and $K_5$ were first obtained, since their initial values were unknown at the time. Initial values for calibrating parameters $L$, $Y$, and $\phi\max$ were taken from the literature (for details see Fishman & Génard, 1998).

Sugars found in 'Brigitta' blueberry fruit corresponded to glucose and fructose, which showed little variation in their proportions during the season (averages of 51% and 49%, respectively). A logistic function with five parameters (similar to Equation 14) was used to fit observed sugar concentration as carbon (carbon of hexoses) in order to calculate the relative rate of sugar transformation ($k$ parameter, Equation 6). The fitted curves showed $r^2$ ranging between 0.93 and 0.97, and $p$ values for coefficients ranging between 0.00317 and $2 \cdot 10^{-16}$ (data not shown). Thus, $k$ was calculated solving Equation (6) for every selected treatment ($k(t) = \left[CC_{fruit} \cdot dM_d(t)/dt - dCTS/dt\right]/CTS(t)$, $\text{g·g}^{-1}\cdot^{-1}$). The amount of C as total sugars ($CTS[t]$, g) was obtained by multiplying the fitted curves of sugar concentration and $M_d(t)$ (data not shown). The $dCTS/dt$ and $dM_d/dt$ were calculated by derivation procedure on $CTS(t)$ and $M_d(t)$, respectively. Assuming $k$ as a genotype-dependent parameter (Grechi et al., 2008) the obtained $k$ curves were then plotted against potential explicative variables, of which the interaction of relative growth rate of the dry mass ($RGR = dM_d[t]/M_d[t]dt$) and time (days after bloom) obtained the best fit ($r^2$= 0.78); the significance of empirical parameters is shown in Table 2. The obtained equation was then included in the model (Equation 7).

The parameters taken from the literature were: (1) the constant a of Equation (4), at 0.0273 (Fishman & Génard, 1998); (2) the fruit potassium concentration at 0.006 g·g-1 $M_d$ (Ochmian, Grajkowski, & Skupień, 2010); and (3) the stem water potential ($\psi_s$), which

**Table 2.** List of parameters obtained by independent experiments and model calibration.

| Parameter (± SE) | Meaning and equation |
|---|---|
| $\rho$ = 76.71*** (± 2) cm $h^{-1}$ | Fruit surface conductance; Equation (2) |
| $y$ = 4.24*** (± 0.08), $z$ = 0.7*** (±0.007) | Empirical parameters relating fruit area ($cm^2$) to fruit mass (g); Equation (3) |
| $L$ = 0.11*** (± 0.01) g $cm^{-2}$ $bar^{-1}$ $d^{-1}$ | Conductivity of the composite membrane for water transport; Equation (4) |
| $Y$ = 1.18*** (± 0.28) bar | Threshold value of hydrostatic pressure needed for growth; Equation (8) |
| $\phi\max$ = 0.4*** (± 0.02) $bar^{-1}$ $d^{-1}$ | Maximal cell wall extensibility; Equation (9) |
| $k_4$ = 0.061*** (± 0.002) | Empirical parameters indicating rate of change of cell wall extensibility; Equation (9) |
| $k_1$ = 3*** (± 0.67), $k_2$ = 0.05*** (± 0.003), $k_3$ = 0.6*** (± 0.09) | Empirical parameters fitted for relative rate of transformation of sugars; Equation (6) |
| $k_5$ = 2.35*** (± 0.43) bar | Pressure given by osmotically active solutes other than sugar and potassium |

*** Parameter significance < 0.0001.

was considered as a decreasing function of days after bloom ($\psi_s = -18.2/\left[1 + 15.1e^{(-0.028DAB)}\right]$), based on our own preliminary results (data not shown) and the results of Bryla and Strik (2007).

The statistical method used to estimate parameters and fit curves was performed in the 'R' software (R version 2.11.1). The derivative procedure was also performed with the routines of the 'R' software (Crawley, 2007).

### *Parameter sensitivity and data analysis*

A sensitivity analysis of the parameters estimated via calibration was performed to identify their influence on fresh mass and sugar concentration for each fruit growth stage. A variation of ± 20% was applied to each model parameter, using conditions of medium fruit load in site 1 (conventional culture) for the 2010–2011 season.

Analyses of variance (ANOVA) were performed for all variables obtained from the trials. Means were separated by Tukey's test ($\alpha = 0.05$) using 'R' software (R version 2.14.2), through 'R'-Commander (Rcmdr version 1.8–3).

### *Model goodness-of-fit analysis*

The root of the mean squared error (RMSE), a common criterion used to evaluate models (Wallach et al., 2001), was used for assessing the model goodness of fit on data used for calibration (internal validation) and on independent data (external validation). RMSE is defined as:

$$RMSE = \sqrt{1/n_d \sum (y_i - y*)^2} \tag{15}$$

where $y_i$ is the observed value, $y^*$ the corresponding simulated value, and $n_d$ the number of observed data. The smaller the RMSE compared to the mean of the observed values, the better the goodness of fit. This can be represented through the relative root of the mean squared error:

$$RRMSE = \frac{RMSE}{\bar{y}} \tag{16}$$

where $\bar{y}$ is the mean of the observed values.

## Results

### *Model parameters*

The values of the obtained parameters (except those taken from the literature) are summarised in Table 2.

Surface fruit conductance ($\rho$) was not influenced by varying fruit fresh mass development ($r^2 = 0.086$, $n = 72$, data not shown) and was therefore considered to be constant (76.71 cm $h^{-1}$). The value estimated for $\rho$ was low compared with those reported for peach by Lescourret, Génard, Habib, and Fishman (2001), but was comparable with those described by Jones and Higgs (1982) for apple (14.4–54 cm $h^{-1}$) and by Ben-Yehoshua, Burg, and Young (1985) for oranges (32.7 cm $h^{-1}$).

The allometric relationship between fruit fresh mass and fruit area had an $r^2$ equal to 0.97 with high significance ($p < 0.001$) for the empirical parameters relating fruit area to fruit mass ($y$ and $z$).

On the other hand, the value of cell wall yielding threshold pressure ($Y$) was estimated at 1.18 bar ($p < 0.001$) – in the lower range of $Y$ values reported for a variety of plant tissues (Bradford & Hsiao, 1982; Green & Cummins, 1974; Green, Erickson, & Buggy, 1971), which range from 1 to 9 bar.

The value of maximal extensibility of the cell wall, $\phi\,\max = 0.4$ bar$^{-1}$ d$^{-1}$ ($p < 0.001$) was similar to the values reported by Cosgrove (1985) for peas (*Pisum sativus* L.; 0.192–0.576 bar$^{-1}$ d$^{-1}$) and used by Fishman and Génard (1998) for peaches (0.24 bar$^{-1}$ d$^{-1}$), but 10 times lower than the value reported for *Mangifera indica* L. (4.08 bar$^{-1}$ d$^{-1}$) by Lechaudel, Vercambre, Lescourret, Normand, and Génard (2007). Thus, cell wall extensibility rapidly dropped during the first 45 *DAB* and reached zero around 85 *DAB*, which was close to fruit ripening in all treatments (Figure 1).

Hydraulic conductivity of the membrane separating the stem and fruit compartments ($L$) was estimated to be equal to 0.11 g cm$^{-2}$ bar$^{-1}$ d$^{-1}$ based on a composite membrane area-to-fruit area ratio of 0.0273 (Fishman & Génard, 1998). This result is lower than values obtained by Steudle, Murrmann, and Peterson (1993) for maize roots (2.33 g cm$^{-2}$ bar$^{-1}$ d$^{-1}$) but comparable with those reported by Nobel (1974) for plant membranes (0.48 g cm$^{-2}$ bar$^{-1}$ d$^{-1}$).

We solved Equation (6) to assign values to the relative rates of sugar transformation and to analyse their seasonal variation from full bloom onwards (Figure 2).

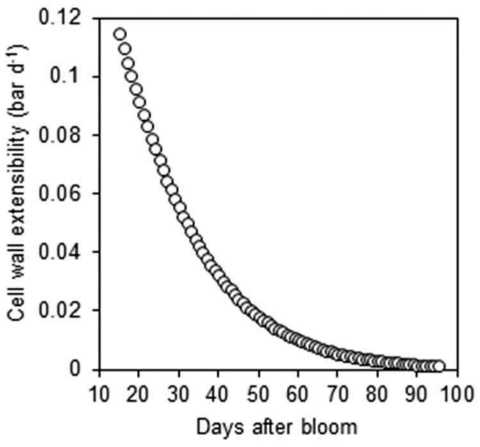

**Figure 1.** Temporal dynamic of the cell wall extensibility ($\phi$) of 'Brigitta' blueberry fruit.

As expected, the relative rates of synthesis of compounds other than sugars (e.g. acids, starch, structural carbon, and proteins) tended to decrease through the season, which led to an increasing sugar concentration in the fruit. In every case, we found a peak (between 55 and 65 *DAB*) followed by a stable decrease in sugar accumulation rate (Figure 2). As mentioned, the interaction between *RGR* and *DAB* (Equation 7), obtained the best fit ($r^2$ = 0.78) for $k$ values compared to other potentially explanatory variables (e.g. only *DAB*), which allowed assessing the effect of fruit growth on sugar accumulation.

## Evaluation and simulation of fresh mass and sugar concentration

A comparison of the seasonal changes of measured and predicted fruit fresh mass and sugar concentration of the treatments used for calibration is depicted in Figure 3.

The double-sigmoid pattern of blueberry fruit growth was successfully simulated by the model (Figure 3(a)–(c)), with RMSE and RRMSE values for internal validation ranging between 0.05–0.12 g and 0.068–0.11, respectively. The final fresh mass was

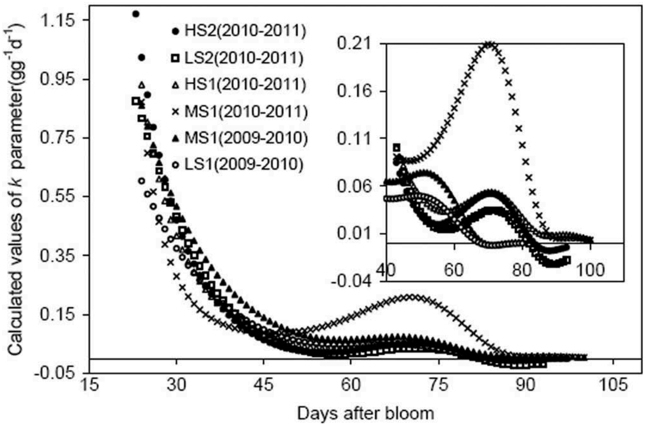

**Figure 2.** Temporal variations in calculated relative rates (g g$^{-1}$ d$^{-1}$) of carbon transformation as sugar for the synthesis of compounds other than sugars through the season for 'Brigitta' blueberry. Letters represent fruit load treatments (H = high fruit load, M = medium fruit load, L = low fruit load), and sites (S1 = site 1 and S2 = site 2). The productive season is shown in parentheses; the inset shows a zoom of the same curves.

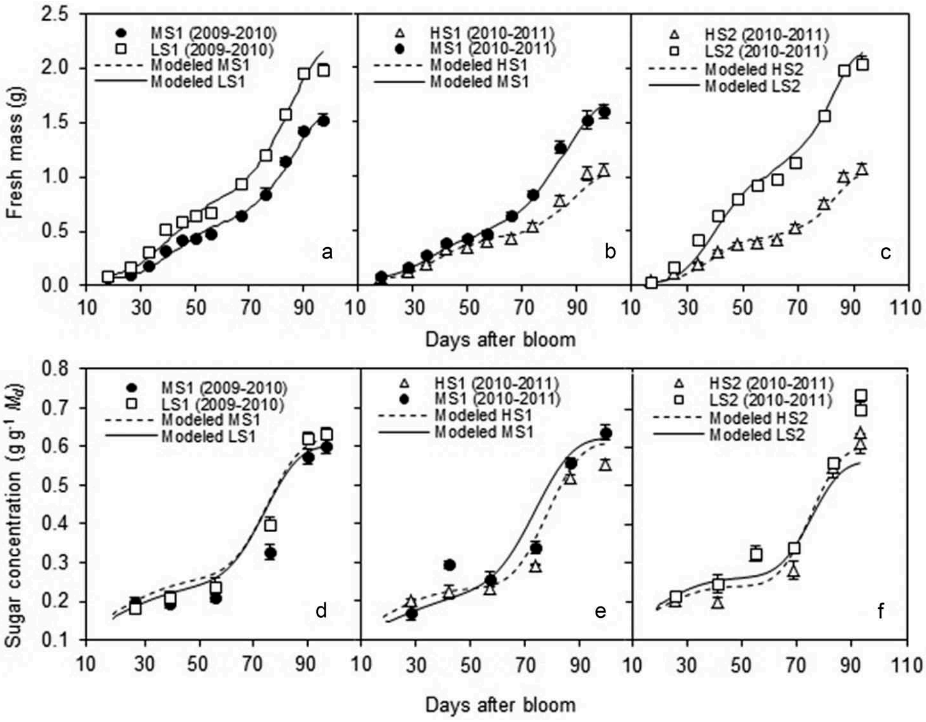

**Figure 3.** Seasonal changes in measured (symbols) and simulated (lines) fruit weight and sugar concentration per unit dry mass ($M_d$) of 'Brigitta' blueberry fruits as affected by different fruit loads, culture systems, and seasons. Letters stand for different fruit loads (H = high fruit load, M = medium fruit load, L = low fruit load) and sites (S1 = conventional culture, S2 = organic culture). The evaluated season is shown in parentheses, while bars represent standard error.

slightly overestimated for treatments with low fruit load, and slightly underestimated for treatments with high load. Medium fruit load treatments were the most correctly fitted. A significant effect ($p < 0.05$) of the treatments on final fresh mass of the fruit was observed, with means of 1.07 g for high, 1.55 g for medium, and 1.97 g for low fruit load. There was no significant effect of culture systems and productive seasons on mean fruit mass under a similar fruit load.

Sugar concentration was acceptably simulated by the model for all treatments, seasons, and culture systems (Figure 3(d)–(f)). The simulations showed values for internal validation of 0.03–0.068 g $g^{-1}M_d$ and 0.07–0.18 for RMSE and RRMSE, respectively. Nevertheless, marked errors were observed in some treatments: overestimations in MS1 (2009–2010) between 35 and 75 *DAB* (Figure 3(d)) and HS1 (2010–2011) at harvest time (Figure 3(e)), and underestimations in HS2 and LS2 (2010–2011) at harvest time (Figure 3(f)). The measured sugar concentration tended to drop when fruit load increased, reaching statistically significant differences ($p < 0.05$), especially in the final values of site 2 (Figure 3(f)). No significant effect of productive seasons (Figure 3(d), (e)) on mean sugar concentration for the same fruit load treatment was found. In contrast, at harvest, a significant effect of culture system was observed for the same fruit load treatment, with HS2 (2010–2011) and LS2 (2010–2011) exhibiting about 10% higher sugar concentrations than HS1 (2010–2011) and LS1 (2009–2010).

The external validation of fruit fresh mass, under the tested conditions, showed an RMSE and RRMSE of 0.057 g and 0.081, respectively. The values were distributed alongside the 1:1 line, although there was some tendency to overestimate the final mass, which can be seen for the highest values of each treatment which exceed the 1:1 line (Figure 4(a)). Values of 0.065 for RMSE and 0.17 for RRMSE were calculated for external validation of simulated sugar concentration (Figure 4(b)). In general, the model tended to underestimate for higher values of sugar concentration and overestimate for intermediate values.

### Simulation of water relations and fluxes

Simulated osmotic and turgor pressures and fruit water potential followed a similar seasonal pattern in all treatments, with increasing fruit loads exhibiting a slight trend towards lower values, almost throughout the season (Figure 5).

The lowest differences in the predicted values of these variables were found when low and medium fruit loads were compared (Figure 5(a), (d), (g)). Due to the close relationship between osmotic pressure and sugar concentration, lower values of osmotic pressure during the first phase of fruit growth were predicted. From about 80 *DAB*, the increasing osmotic pressure reached a plateau for all treatments, exhibiting a trend to lower osmotic pressures when plants had lower fruit loads, which is explained by model results showing slightly lower sugar concentrations with decreasing fruit load (Figure 3).

The turgor pressure was low before the third phase of fruit growth (Figure 5(d)–(f)), indicating that the cell wall did not restrict fruit growth, which is concordant with a still relatively high cell wall extensibility estimated for that growth phase (Figure 1). No important differences in turgor pressures between the treatments were observed. Similar to osmotic pressure, from about 80 *DAB* there was a slight tendency towards higher turgor pressures for higher fruit loads.

The increasing differences between osmotic and turgor pressures with time led to a concomitant drop in fruit water potentials, regardless of season and culture system (Figure 5(g)–(i)). On the other hand, the

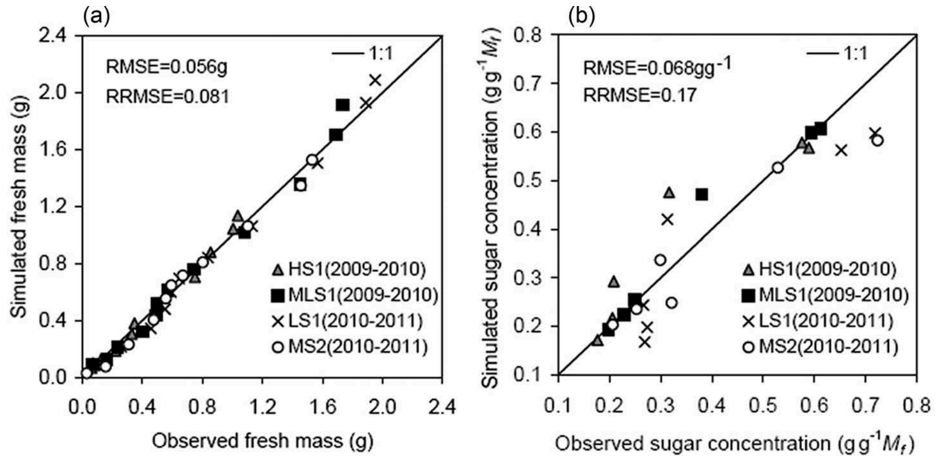

**Figure 4.** Simulated values of fruit fresh weight (a) and simulated values of sugar concentration (b) of blueberry cv. 'Brigitta' plotted against corresponding observed values assessed in seasons 2009–2010 and 2010–2011 from the high fruit load in site 1 (HS1), medium low load in site 1 (MLS1), low fruit load in site 1 (LS1) and medium fruit load in site 2 (MS2). The root mean squared error (RMSE), relative mean squared error (RRMSE), and 1:1 lines are indicated in the figure.

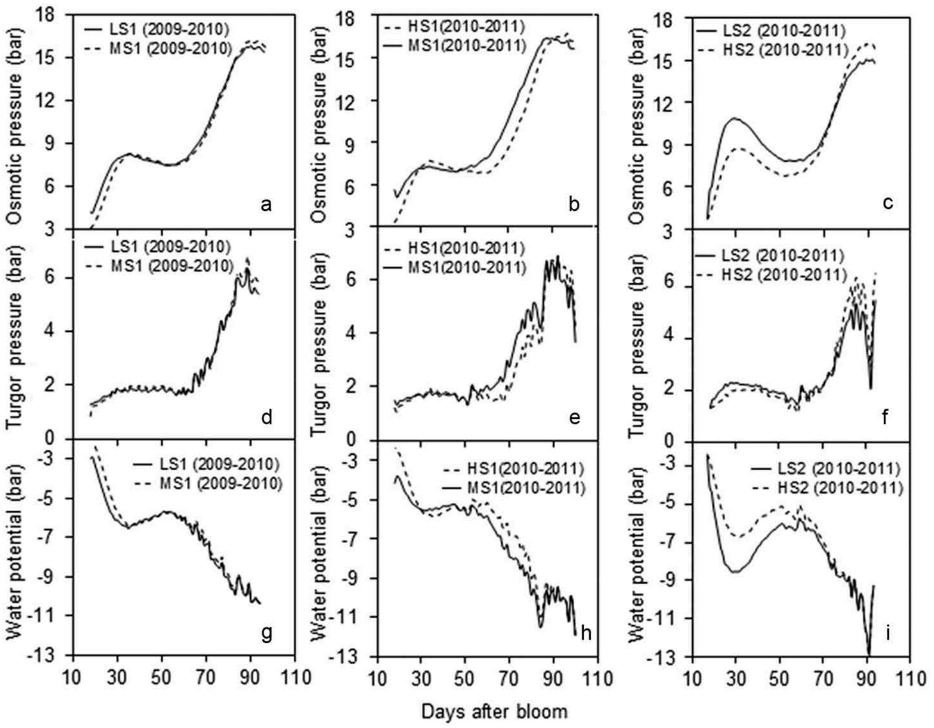

Figure 5. Seasonal changes in simulated pressures involved in blueberry cv 'Brigitta' fruit growth under different fruit loads, sites and seasons. Letters indicate different fruit loads (H = high fruit load, M = medium fruit load, L = low fruit load) and sites (S1 = conventional culture, S2 = organic culture). The productive season is shown in parentheses.

gradual increase in air temperature paralleled by a decrease in air humidity through the seasons resulted in increasing transpiration rates (Figure 6(d)–(f)).

In the highest fruit load treatments, water influx decreased by about 47%, whereas transpiration rates dropped by 36% over the growth period, as compared to the water influx and transpiration of the lowest fruit load condition, independently of year and culture system conditions (Figure 6). The water balances reached two marked peaks (Figure 6(g)–(i)), which are related to the changes in growth phases. Thus, maximal water uptake rate in the highest fruit load treatments was almost twice that of the lowest fruit load treatments.

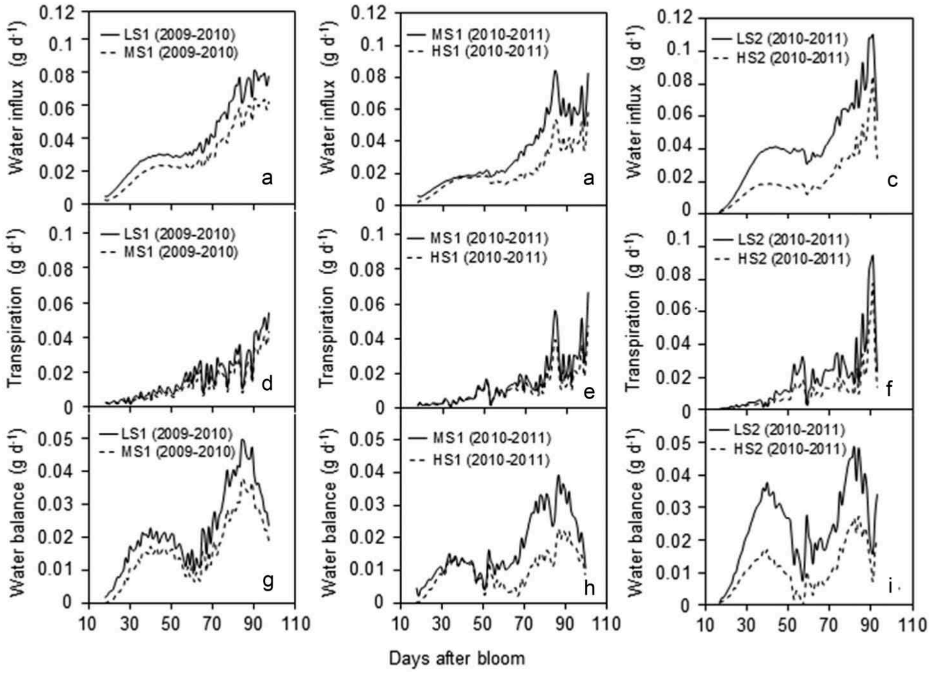

Figure 6. Seasonal changes in simulated water relationships involved in blueberry cv. 'Brigitta' fruit growth under different fruit loads, sites, and seasons. Letters indicate different fruit loads (H = high fruit load, M = medium fruit load, L = low fruit load) and sites (S1 = conventional culture, S2 = organic culture). The productive season is shown in parentheses.

### *Parameter sensitivity analysis*

The model showed different degrees of sensitivity according to the evaluated parameters and the growth phases (Table 3).

The threshold value of hydrostatic pressure ($Y$) did not induce important alterations in fresh mass, whatever the growth stage. In contrast, variations of hydraulic conductivity ($L$) induced important changes in fresh mass at the first two growth phases. At harvest time, the model was highly sensitive to parameter $K_4$ (used to estimate the rate of decrease of cell wall extensibility; Equation 9) but was only weakly sensitive to $\phi \max$. Regarding the empirical parameters relating fruit area and fruit fresh mass ($y$ and $z$; Equation 4), the largest variations in fresh mass were observed in the first two stages of growth, which did not induce dramatic changes in the final weight. In the same way, no big changes were observed by variations of fruit surface conductance.

Growth of fresh mass was also very sensitive to variations of coefficients involved in the relative rate of carbon transformation in compounds other than sugars (Equation 7). The $K_2$ coefficient showed a great influence on fruit fresh mass and sugar concentration, mainly during the second growth period. The $K_3$ coefficient was not as influential as the previous one during the first and second phases of fruit growth, although, at harvest, the evaluated variations induced one of the strongest changes in fruit fresh mass and sugar concentration. The $K_1$ parameter led to similar variations in fruit mass in each stage of growth. This parameter, on the other hand, induced high variations in sugar concentration mainly at the first and second phases of fruit growth. Variations of parameter $K_5$ (representing osmotically active solutes other than sugars and potassium), caused significant changes in the fruit fresh mass, especially during the first and second phases of fruit growth.

**Table 3.** Analysis of model sensitivity for parameters obtained by model calibration and by independent measurements.

| | | Fresh weight (%) | | | Sugar concentration (%) | | |
|---|---|---|---|---|---|---|---|
| | | Growth phase | | | Growth phase | | |
| Parameters | Variation (%) | I | II | III | I | II | III |
| $Y$ | +20 | −2.9 | −3.6 | −1.6 | | | |
| | −20 | 3.0 | 3.7 | 1.8 | | | |
| $L$ | +20 | 7.6 | 8.5 | 4.5 | | | |
| | −20 | **−10.2** | **−12.3** | −6.7 | | | |
| $\phi$ max | +20 | 0.9 | 1.2 | 4.0 | | | |
| | −20 | −1.3 | −1.8 | −5.7 | | | |
| $k_4$ | +20 | −0.3 | −1.7 | **−21.8** | | | |
| | −20 | 0.3 | **−16.6** | **15.2** | | | |
| $\rho$ | +20 | −2.3 | −3.7 | −2.7 | | | |
| | −20 | −2.3 | 3.6 | 2.7 | | | |
| $Y$ | +20 | 4.1 | 6.2 | 2.3 | | | |
| | −20 | −4.4 | −8.0 | 3.4 | | | |
| $Z$ | +20 | −6.6 | −6.7 | −0.2 | | | |
| | −20 | 7.0 | 5.8 | 0.2 | | | |
| $k_1$ | +20 | −6.0 | −8.5 | −4.6 | **−15.8** | **−14.0** | **−5.3** |
| | −20 | 7.1 | 9.5 | 8.9 | **16.0** | **14.3** | 5.6 |
| $k_2$ | +20 | 8.3 | **14.4** | 8.7 | **20.1** | **23.7** | 9.5 |
| | −20 | −9.1 | **−18.5** | **−11.0** | **−28.8** | **−38.4** | **−13.9** |
| $k_3$ | +20 | 2.0 | 7.2 | 8.9 | 7.6 | **14.5** | **11.5** |
| | −20 | −2.2 | −8.9 | **−14.8** | −9.3 | **−21.2** | **−22.9** |
| $k_5$ | +20 | 5.8 | 7.2 | 3.4 | | | |
| | −20 | −6.0 | −7.3 | −3.3 | | | |

The variations are expressed as a percentage of the reference value. Variations in fruit fresh mass or sugar concentration exceeding 10% are bolded.

## Discussion

Fruit quality has become an increasingly important aspect of fruit production, and models are a powerful tool to understand the key processes involved in the control of quality through the production season. Our blueberry fruit growth model was based on a theoretical approach to water fluxes and cell growth in fruit, which was originally developed for peach fruit by Fishman and Génard (1998). This model with modifications has also been tested in several other fruits (Bar-Tal et al., 1999; Lechaudel et al., 2007; Liu et al., 2007; Quilot, Génard, Lescourret, & Kervella, 2005). Our model adaptation to blueberry fruit focused on cell wall extensibility and sugar uptake, processes for which the model was very sensitive (Table 3) and, for the tested conditions, rendered successful simulations that were validated internally and externally under several contrasting trial conditions with a common set of parameters. Although fruit dry mass and soluble sugar content in this study were obtained from empirical observations and are therefore valid only for the tested conditions, this model adaptation confirms the generalizability of the original model regarding the dynamics of fruit water through the whole growth period and its suitability for blueberry.

The effect of fruit load on fruit growth and sugar content has been widely studied in fruit crops (e.g. Léchaudel, Joas, Caro, Génard, & Jannoyer, 2005, Souty, Génard, Reich, & Albagnac, 1999; Wünsche, Palmer, & Greer, 2000). In blueberry, the management of fruit load via pruning intensity and/or reproductive bud removal has implications for fruit fresh mass (Strik et al., 2003), which we confirmed and simulated under the studied conditions, regardless of culture system and year (Figures 3(a)–(c) and 4). We found that the chosen year and culture system had no significant effect on mean fruit fresh mass for the same fruit load, indicating that, in these circumstances, fruit growth mainly depends on its sink strength, which is driven by fruit load, provided that water and fertiliser are not limiting factors (e.g. Grossman & DeJong, 1995).

Year, culture system, and fruit load had no dramatic effect on sugar concentrations throughout the season. However, the slight differences found, especially at harvest, for both culture system and fruit load, were not adequately reproduced by the model, which tended to underestimate the highest values and overestimate the lowest ones, resulting in a lower goodness-of-fit as compared with fruit fresh mass (Figure 4). As sugar concentration and osmotic pressure are closely related, the model's inaccuracy resulted in lower osmotic pressure at lower fruit loads towards the end of the growth period (Figure 5(a)–(c)). Nonetheless, in general, increases in fruit load tended to reduce the simulated fruit osmotic pressure almost throughout the season, which agrees with results found for peaches by Fishman and Génard (1998).

The empirical parameters of $K$ (Equation 7) were highly significant and showed to be very sensitive (Table 3). The inclusion of metabolic activities and environmental variables in sugar modelling should therefore improve the performance of sugar predictions. In fact, our results suggest that compounds other than soluble sugars translocated to the fruit, such as insoluble sugars, might be hydrolysed into soluble sugars, which would be represented by the negative $K$ values found near fruit ripening (Figure 2). Such a situation has been reported in several fruits (e.g. Coombe, 1976; Souleyre et al., 2004). Nevertheless, the inclusion of other metabolic processes would greatly increase the complexity of the model (Génard, Lescourret, Gomez, & Habib, 2003).

According to Steudle, Zimmermann, and Luttge (1977), under conditions in which sugar concentration in fruits remains low and cell enlargement is restricted, low turgor pressures can occur. This was simulated at the two first stages of 'Brigitta' blueberry fruit growth (Figure 6(d)–(f)), where sugars were not concentrated and probably cell division significantly drove fruit growth. Lower sugar concentration during these development stages was previously reported in cultivated blueberry species (Darnell et al., 1994). Increases of the fruit turgor pressure as growth advances are induced by a drop in cell wall extensibility (Liu et al., 2007) and by an increase in osmotic pressure (Génard & Lescourret, 2004), which is consistent with our predictions (Figures 1 and 5). For all treatments, the simulated turgor strongly increased from the beginning of the last phase of growth (Figure 5(d)–(f)), which corresponds to the stage in which cell enlargement determines fruit growth. This result is consistent with the fact that cell enlargement is driven by turgor pressure created by water uptake but, on the other hand, restricted by the extensibility of the cell wall, which was predicted to strongly drop during the cell enlargement period (Figure 1). When the fruits approached ripening, a drop in turgor pressure was observed for all tested situations (Figure 5(d)–(f)), which was partially concomitant with a stabilisation of the osmotic pressure. The falling rates of turgor pressures at fruit ripening have been related to increases in fruit transpiration (Lechaudel et al., 2007; Morandi, Rieger, Corelli, & Grappadelli, 2007), which is in line with our results (Figure 6(d)–(f)). On the other hand, decreasing turgor pressure when fruits are close to ripening has been related to cell wall degradation via pectin methyl esterases and polygalacturonases in blueberry (Proctor & Miesle, 1991).

While the pressure components simulated by the model did not widely differ among the evaluated conditions, water fluxes and their resulting balance showed greater differences (Figure 6). This finding indicates that cell enlargement was not too different among treatments, implying that the differences in fruit mass were mainly induced by cell number. It can, therefore, be hypothesised that source activity during the first stage of fruit growth, when cell division occurs, did not supply enough sugar to the fruits to reach the threshold required for sustaining optimal cell division rates.This has been reported for 'Herbert' highbush blueberry (Godoy et al., 2008) and confirms that variations in fruit size and diameter are primarily facilitated by variation in cell number, which was recently reported for different rabbiteye blueberry genotypes (Johnson et al., 2011).

Other osmotically active solutes and their dynamics might be incorporated in our blueberry model to improve its predictive power. In this respect, we estimated the contribution of other osmotically active solutes to osmotic pressure at 2.35 bars (parameter $K_5$ in Table 2). In the sensitivity analysis, this contribution induced a significant variation in fresh weight mainly during the first and second phases of fruit growth (Table 3), which could be related to the effect of organic acids dominating the osmotic activity during these phases (Ismail & Kender, 1974).

Decreasing stem water potential was used for the simulations, regardless of treatment, growth season, or site. However, it has been reported that fruit load can affect the water potential of leaves and stems (Berman & DeJong, 1996; Guichard, Gary, Leonardi, & Bertin, 2005), hence affecting fruit water uptake. The model may also be improved in this aspect.

## Conclusions

The theoretical framework of the present model enabled us to accurately predict the dynamics of blueberry fruit fresh mass accumulation and, to a lesser extent, fruit sugar concentration, which represent significant commercial quality traits of the fruits. Fruit load mainly affected fruit fresh mass, rather than sugar concentration; in fact, differences in sugar concentration were small, which led to a lower level of prediction accuracy for sugars compared to fruit fresh mass. Our simulations showed that larger fruit fresh mass is mainly controlled by increases in water fluxes rather than pressure differences, which suggests that final fruit weight was not determined by cell expansion. The model implementation gives the first estimates of a set of parameters which govern blueberry fruit growth and sugar dynamics. Provided that measurements of sugar composition and dry mass increment are available, these values, along with the sensitivity analysis presently performed, can be used for improving the analysis of the determinants of blueberry fruit growth and sugar accumulation in response to different management practices and environmental conditions, and may serve as breeding targets for increasing blueberry fruit size and sweetness. An estimation of water potential in the stem and its validation, the incorporation of other osmotically active solutes, and an improvement in the theoretical framework of sugar uptake are the next steps to be taken in order to further develop the model.

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## Disclosure statement

No potential conflict of interest was reported by the authors.

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