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A virtual fruit model to simulate water deficit effects on water and solutes accumulation in the fruit and the consequences on fruit quality

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Abstract

Fruit size and solute composition are quality traits and are mainly determined by soluble sugars, acids, and mineral in concentrations that depend on water and solute translocation and metabolism. Water and sugar translocation depend on transport mechanisms that take place in the vascular system connecting the plant and the fruit. These transports are driven by the fruit water potential, which is determined by the concentrations of solutes in the fruit cell vacuoles. Nevertheless, this concentration determines the fruit osmotic potential itself. Therefore, there is strong feedback between the solutes and water translocation, metabolism, and fruit growth.

Water deficit leads to changes in the water status of plants and therefore affects fruit growth. Understanding links between fruit growth processes and the plant growing conditions can be important to improve agricultural techniques and fruit quality. In this work, we show how we can describe such a complex system through a virtual fruit model, i.e. a process-based model composed of sub-modules that describe fruit growth and sugar and acid metabolism by using biophysical relationships.

The model simulations well predicted the observed fruit size, and solutes concentrations of three cohorts of two commercial cultivars of tomato.

We simulated the model on two scenarios of water deficit and we explored how the biophysical processes involved lead to changes in fruit growth with a focus on the role of sugar and acid accumulation and metabolism.

The simulation results suggested that water deficit conditions could increase solutes concentrations and that dilution and osmotic potential changes are important variables to evaluate water deficit effects on fruit solutes content. The presented virtual fruit model is a promising tool to untangle the complex processes that involve fruit growth and to simulate environmental conditions for predicting response in fruit growth processes and their effect on fruit quality.

Keywords

Acid, dilution, process-based, quality, shortage, size, sugar, , tomato

INTRODUCTION

Fruit quality at harvest can be described by agronomic variables that consider size and chemical composition. The increase in fruit size increases crop yield, and the concentrations of sugars and acid determine fruit taste, which is important for fruit quality (Guichard et al., 2001). Fruit size and fruit chemical composition depend on the accumulation of water and sugars in the organ during the fruit growth and metabolism (Matthews and Shackel, 2005). The water and solutes accumulation occur by their transfer between the fruit and the plant. The concentrations of fruit solutes depend on the solutes and water mass and on the metabolism in the fruit cells. Such concentrations determine the fruit osmotic potential, which together with the fruit pressure potential form the fruit water potential. These variables drive the transport of water and solutes in the fruit and plant vascular networks. Therefore, there is strong feedback between the transport of solutes and their metabolism. Water deficit can be a severe stress for the plant leading to loss of yield. However, it can be controlled for obtaining higher fruit quality (Chen et al., 2013). Understanding the mechanisms underlying fruit growth is then crucial.

The problem associated with such a complex system can be simplified and analyzed by representing the water and solutes transport mechanisms through mathematical process-based models. Such powerful tools have been used for linking agricultural practices and the biophysical mechanisms of fruit growth in many studies on different species (Baldazzi et al., 2013; Hall et al., 2013; Zhu et al., 2019). The metabolisms and transport of the main fruit solutes were described by process-based models. However, these different processes, which all determine fruit growth functioning have not yet been linked. In this work, we present a model of “virtual fruit”. It describes fruit growth by linking the descriptions of sugars and acids accumulation in the fruit cell vacuole, using the fruit osmotic potential as the linking variable. This model has exogenous variables that regard the plant surrounding environment and allows the simulation of the response of fruit solutes transport and metabolism in response to different water deficit conditions.

In this work, we present the model performance in simulating the dynamics of the concentrations of solutes during fruit growth for two commercial tomato cultivars. We then analyze the response of the model to different levels of water deficit, discussing the behavior of some relevant biophysical variables related to fruit growth and defining the perspective of the model conception.

MATERIALS AND METHODS

Plant material

Tomatoes (*S. lycopersicum*) of two commercial cultivars (Bellastar and Sassari) were grown in a greenhouse from March to the end of October 2018 with local commercial cultural practices and two different shoot densities (2.5 shoots per squared meters and 3.6 shoots per square meters, i.e. low and high density respectively). The flowering of trusses was observed at 3 periods, constituting then 3 cohorts of fruits. The measures were done on 20 fruits for each cultivar and modality. Fresh weight, dry weight, and soluble sugars, acids, and potassium concentrations were measured. Each week, 20 fruits of each cultivar were harvested and their fresh weight was measured Half of the fruit was dried in a ventilated oven to estimate dry matter content. The pericarps of the other fruits were stored at -80°C for biochemical analysis. The freeze-dried samples were weighted and powdered. Soluble carbohydrates and organic acids were determined after extraction with a water-methanol-chloroform mixture as in Gomez et al. (2002). sugars were measured using a high-performance liquid chromatography, Organic acids were measured using an HPLC system. Starch was measured using an enzymatic micro-plate assay as described by Gomez et al. (2007). Minerals were measured using a portable X-ray fluorescence spectrometer (P-XRF).

The virtual fruit model

The model describes the dynamics of water, soluble sugars, and acid concentrations in the fruit cells during fruit growth. We used as the main assumption that the fruit is constituted by a big cell and that the vascular system of the fruit is connected to that of the plant through xylem and phloem vessels (Liu et al., 2007). Moreover, we consider that the solute storage is in the vacuole of the fruit cells. The virtual fruit model is divided into sub-modules as depicted in Figure 1.

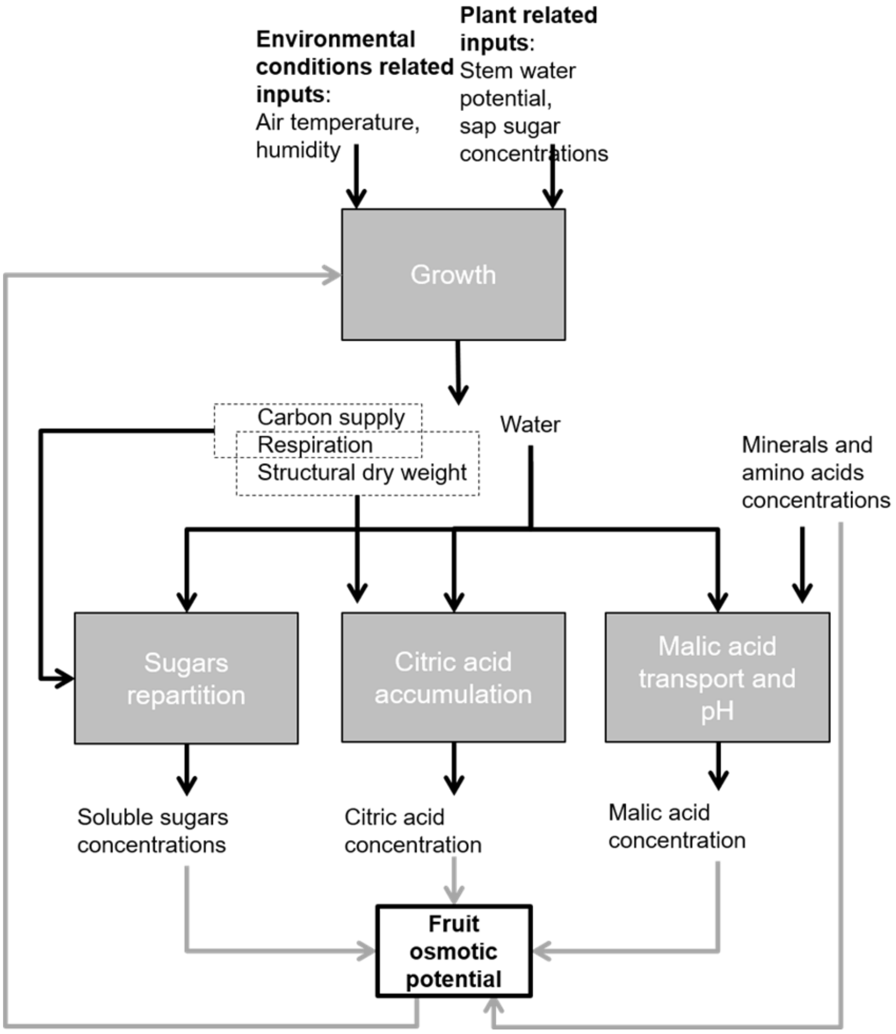

Figure 1: Virtual fruit model composed of different sub-modules describing different processes of fruit growth.

The growth sub-module describes fruit growth with the model used by Hall et al. (2013). The fruit dry and fresh mass variations are described by the balance of water and sugars in the fruit cells. The input of this module is composed of the stem water potential and concentration of sugars, from which we compute the balance of water and sugar. The water translocation is represented by a model which contains parameters of conductance and water potentials that drive water transports and parameters regarding the rates and mechanisms of sugar uptake processes of the fruit cells, with an explicit distinction between symplastic (mass flux) and apoplastic (active + passive flux) processes. The mechanical limitation of the expansion of the cells is represented by Lockhart's law which describes the plastic behavior of the cellular expansion. The fruit water potential Ψ_W is the sum of the two contributions of the (hydrostatic) pressure potential Ψ_P and the osmotic potential Ψ_π, i.e. $\Psi_W = \Psi_P + \Psi_\pi$. This model differs from the previous models of the fruit growth because we use the dynamics of the measured or simulated values of the molar concentration of each solute *i*, C_i, to compute the osmotic potential as $\sum_i -(RTC_i)$ i.e. the sum of the portions of osmotic potential due to each solute *i* (being R and T the universal gas constant and the absolute temperature respectively). The dynamics of the concentration of the soluble sugars, the citric and the malic acid along fruit growth are simulated by each sub-module. Each sub-module uses outputs of the growth module as input, with water mass as the common input variable. The dynamics of the concentration of amino acids, potassium, and H3O+ cations were estimated from the measurements of minerals and pH.

The sugar repartition sub-module contains the model of Luo et al. (2020). This module simulates the soluble sugars and the starch concentrations in the vacuole using the simulated carbon supply of the fruit, and the fruit respiration as input.

The citric acid sub-module is a simplified description of the Krebs's cycle made following Etienne et al. (2015). It uses the simulated fruit respiration and fruit's structural dry weight as input, and predicts the concentration of citric acid in the vacuole.

The malic acid sub-module is a description of the functioning of the malic acid transport from the cytosol to the vacuole as proposed in Lobit et al. (2006) and Etienne et al. (2014). This module uses as input the measured concentrations of minerals and concentrations of amino acids that were estimated, and predicts the malic acid concentration in the vacuole. This sub-module also simulates the vacuolar pH, that drives the malic acid transport from the cytosol to the vacuole. We estimated the amino acids hypothesizing that glutamine was the only amino acid in the fruit solution and that its accumulation was directly proportional to the inflow of dry mass in the fruit.

Model inputs and parameters choice

The model input (Figure 1) was taken from measurements of the air temperature and humidity in the greenhouse, and the sap sugar concentration input was taken from literature values provided by Liu et al. (2007). The stem water potential input was set at a constant value between 18.00 and 06.00 h. Then, it was set at a sinusoidal behavior (period of 12h) between 06.00 and 18.00 h, with a fixed maximum value at noon. Some values of the sub-modules parameters were chosen using literature-based values (Liu et al., 2007 for the growth sub-module; Luo et al, 2020, Etienne et al, 2014, and Etienne et al., 2015 for the sub-modules related to sugars, citric, and malic acid). A model calibration procedure was used to estimate the values of the remaining parameters.

The calibration of the sugar, citric acid, and malic acid sub-modules was made to minimize the error made by the model in the simulation of the observed data of soluble sugars, starch, citric, and malic acid concentrations for the three cohorts of the two cultivars grown in the two different densities of shoots. We derived the input variables of the sub-modules from the observed fruit fresh weight, dry weight, and concentrations of soluble compounds. The estimated parameters were then used in the global model to calibrate the parameters of the growth model minimizing the prediction error of the simulation of the fresh and the dry mass of the fruit.

We calibrated 4 parameters of the sugar sub-module related to the rates of conversion of the carbon uptake to the carbon transformed in soluble sugars, starch, or carbon for the synthesis of new metabolites and structural compounds, 8 parameters of the citric acid sub-module related to the rates of reaction of the considered Krebs's cycle reactions, 1 parameter related to the free energy variation of the ATP hydrolysis of the malate sub-module.

The values of the calibrated parameters of these sub-modules were used for the calibration of the growth model. For this last sub-module, we calibrated 8 parameters: 3 parameters related to the conductance of the vessels in the xylem and the phloem connecting the plant to the fruit and the pedicel, 3 parameters related to the active uptake of sugars in the fruit cells, one parameter related to the cell wall extensibility, and one parameter related to the process of the gradual interruption of the symplastic transport during fruit growth. The calibration procedure was performed for each cultivar and consisted in finding the best solution of a minimization problem in which we minimized given sets of cost functions for each of the sub-modules simulation. The generic cost function we used was RMSE. Given two time series of N simulated and observed values S_t and O_t for the time t of a variable v, we define RMSE corresponding to the variable v as follows:

$$RMSE_v = \sqrt{\frac{1}{N}\sum_{t=1}^{N}(S_t - O_t)^2} \qquad (1)$$

The method used for minimizing the cost functions was NSGA-II (Deb et al., 2002). The result of NSGA-II is a set of Pareto-dominant solutions, i.e. sets of parameters that give the solutions belonging to the Pareto front. We used a rule for choosing the best solution among all the Pareto-dominant solutions. In the case of the malic acid prediction, we did not use any rule because there was only one minimized cost function. In Table 1, we present the minimized objectives for each sub-module and the rule used for choosing the best solution, for each cultivar.

Table 1: Cost functions minimized in the model calibration procedure and rule of selection of the best solution among the Pareto-dominant solutions

Module	**Minimized RMSEs**	**Rule of selection**
Sugar	RMSEs of the soluble sugars and the starch concentrations predictions, averaged on the 2 treatments and the 3 cohorts. RMSEsol, RMSEsta	Solution with the minimum average between RMSEsol and RMSEsta
Citric acid	RMSEs of the citric acid concentrations predictions, averaged on the 2 treatments, a value for each cohort. $\text{RMSEcit}_{\text{cohort1}}$, $\text{RMSEcit}_{\text{cohort2}}$, $\text{RMSEcit}_{\text{cohort3}}$	Solution with the minimum average between $\text{RMSEcit}_{\text{cohort1}}$, $\text{RMSEcit}_{\text{cohort2}}$, $\text{RMSEcit}_{\text{cohort3}}$
Malic acid	RMSE of the malic acid concentrations, averaged on the 2 treatments RMSEmal	no rule of selection with only one RMSE
Growth	RMSEs of the fresh and dry weight predictions, averaged on the 2 treatments and the 3 cohorts. RMSEfresh, RMSEdry	Solution with the minimum average between RMSEfresh and RMSEdry

Water deficit scenarios

The virtual fruit is a tool to simulate fruit growth under predetermined environmental conditions. These conditions can be simulated by changing the model input. To simulate water deficit scenarios, we modulated the stem water potential given as input to the model (Figure 1). For the simulation of different levels of water deficit, we used the minimum and maximum values of water potential measured in the Ph.D. thesis work of Najla (2009) on tomato plants. The scenarios chosen for the model simulations are shown in Table 2. We assumed that the water potential behavior was as it has already been described in the section Model inputs and parameters choice.

Table 2: maximum and minimum water potential values used for providing the stem water potential input of the model

Scenario	Maximum water potential (MPa)	Minimum water potential (MPa)
Low WD	-0.2	-0.5
Medium WD	-0.3	-0.6
High WD	-0.4	-0.7

The growth module was simulated with the parameter sets obtained via the model calibration and the input values used for the model simulations in the calibration step, with all the sub-modules, and modifying the stem water potential given as input, to analyze the responses of some variables of interest. We used the resulting simulations of the 2nd cohort of the cultivar Sassari, which gave a more satisfying fit. We studied the variation of some output variables that concern fruit quality and the processes that are linked with them, i.e. the dry matter content, the sugar and acid concentrations and contents on dry weight-base, the sugar-acid ratio, and the pH.

RESULTS AND DISCUSSION

Model fitting results

We report in Figure 2 that the model simulations conformed to the observed fresh weight, dry weight, soluble sugar, and acids concentrations for the cultivar Sassari. The simulations of the solutes concentrations and fruit growth variables for the two varieties and the two treatments produced similar results. We thus decided not show both in this short presentation of the model performance. These results concern the best simulation obtained with the complete model, that was chosen using the criteria in Table 2.

The simulated dynamics of the fruit dry and fresh mass and solutes concentrations represented with good accuracy the observed dry weight accumulations and the fresh weight growth, even though higher values of the cohort 2 were not well estimated.

Sugars concentration behaviors were not so different for the three cohorts suggesting that some other effects would have to be considered in the sugar module e.g. the fruit water content effect on sugar metabolism (Chen et al., 2020), which was not used for simplifying the parameterization of the model.

Acids dynamics were well represented but overestimated in the case of citric acid. The module of malic acid is the only module that is solved as a steady-state model, i.e. does not depend on initial conditions. We can see that the malic acid mode succeeded in showing the ranges of variation of this variable given the estimated amino acids and the observed potassium concentrations as input. This suggested that the pH + malic acid sub-module was working correctly and that the amino acids inflow might have been well estimated with our method.

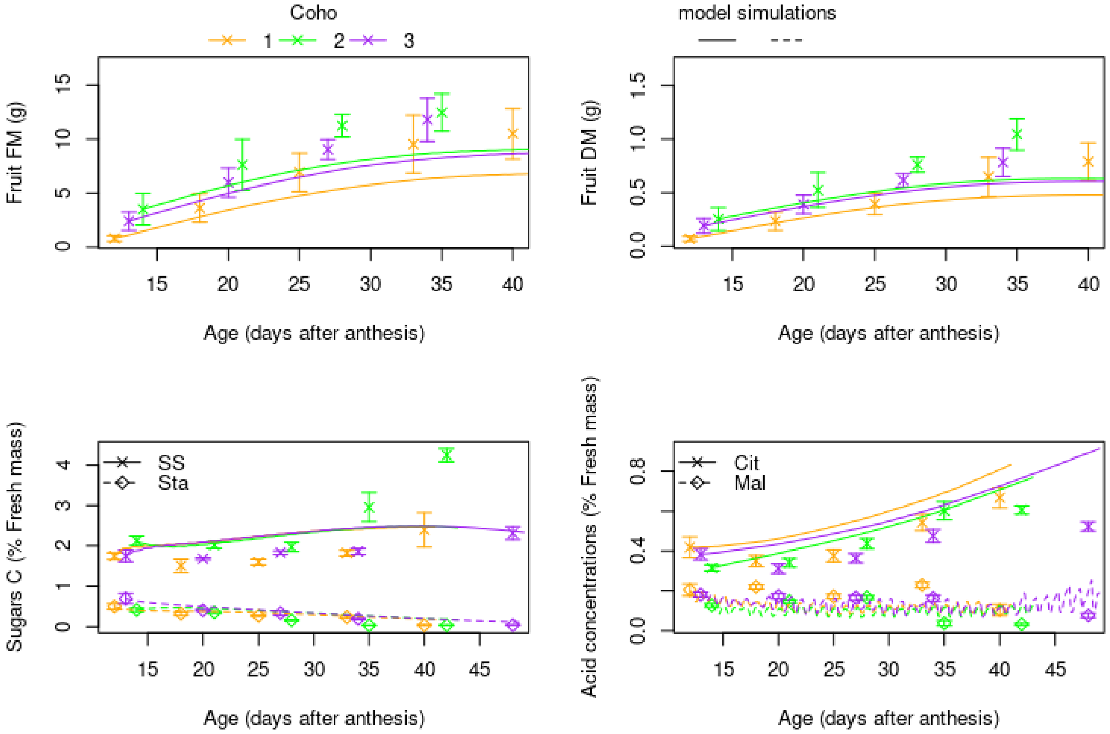

Figure 2: Model simulations of fruit fresh and dry mass, concentrations of sugars and acids (soluble sugars SS, starch, citric acid, and malic acid) during the fruit growth of three cohorts of fruits of the cultivar Sassari. Points represent the observations, lines represent the model simulations.

Overall, the model performed well, despite the use of the mineral concentrations and the initial values of the sub-modules as differences between the three cohorts. We also showed that the fruit osmotic potential and, thus, fruit water potential gave good results if used as a linking variable in organ growth models, as also proposed by De Swaef et al., (2022).

Water deficit scenarios

In figure 3 we show the how the three different scenarios of water deficit used as inputs to the model affected the simulated dynamics of the fruit dry and fresh weight, the concentrations of soluble sugars and acids on a fruit fresh weight and dry weight basis, the sugar import and respiration fluxes, and the fruit osmotic potential.

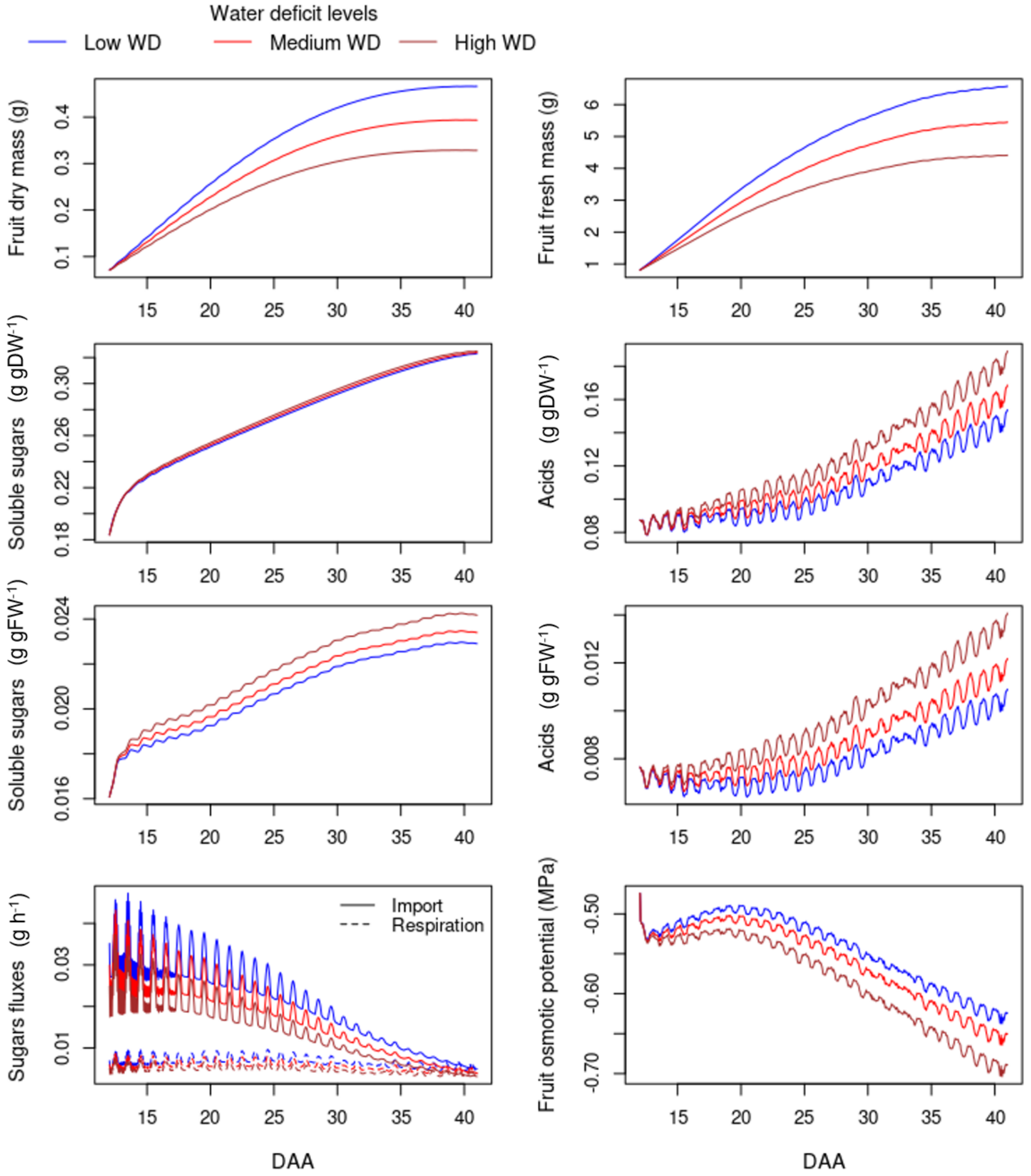

Figure 3: Simulated dynamics of fruit dry and fresh mass, soluble sugars and acids concentrations on a dry and fresh weight basis, sugar fluxes, and fruit osmotic potential for different water deficit scenarios, during the fruit growth (DAA is Days After Anthesis). The blue line represents the reference simulation (cultivar Sassari, cohort 2), the red lines represent the medium water deficit scenario, and the brown lines represent the high water deficit scenario. The simulated sugar fluxes are shown separately as the overall sugars imported by the fruit from the bearing shoot and the sugars lost by respiration.

The simulations showed that water deficit strongly reduced the fruit dry mass, fresh mass, and increased acids concentrations, while it did not modify the soluble sugars concentrations on either a dry and fresh weight basis.

Sugar fluxes and the fruit osmotic potential were reduced by a higher water deficit level. As reported in Hou et al. (2020), studies on water deficit effects on tomato growth and quality had different conclusions concerning changes in sugars and organic acids concentrations after the application of water deficit. Our model suggests that sugar concentrations are not strongly affected by water deficit as was also concluded by Ripoll et al. (2016), and Chen et al. (2014).

Acid concentrations were increased by water deficit as found in the same studies. The process of the accumulation of solutes has been associated with the changes in fruit cells' osmotic potential and osmotic regulations that would permit the solutes to be imported by the cells despite the low water potential in the stem vessels (Hou et al., 2020).

In our simulations, the concentrations of solutes increased and fruit osmotic potential decreased for increasing water-deficit levels. However, this did not increase the solutes imported into the fruit, under the model hypothesis that sugars are the main solute in the phloemic tissues of the stem and the fruit vascular system. Moreover, the dry and the fresh mass of the fruit had a strong suppression for the simulated levels of water deficit, changing the concentrations of solutes and, by consequence, the fruit osmotic potential. Therefore, the increasing concentrations of solutes could be related to dilution effects, rather than to higher solutes import.

These results suggested that dilution of solutes in the fruit cells could have an important role in fruit quality, as also suggested by Chen et al. (2014) and Génard et al. (2014). Interestingly, the acid concentrations increased with a higher level of water deficit, while the simulated accumulation of citric and malic acid decreased (results not shown). This further highlights the conclusion that the effects of water deficit depend on how the condition affects the dry and water mass loss and the solutes accumulation processes, rather than only on how solutes concentrations affect the fruit osmotic potential.

CONCLUSIONS

In our work, we presented a virtual fruit model, i.e. a process-based model of fruit solutes transport and metabolism, that we used to describe the water and solutes accumulation in tomato fruit. We explored the responses of the fruit model to different scenarios of water deficit. The model simulations suggested that water deficit could increase solute levels, and that dilution effects played an important role in determining solute concentrations. Moreover, the simulations showed that water deficit could limit solute import in the fruit, despite the lower fruit osmotic potential, suggesting the necessity of further studying the effects of the osmotic regulation.

In future studies, the model will be calibrated and validated on measurements made for controlled irrigation vs water deficit conditions. This modeling approach could be used to disentangle the transport metabolic processes that drive fruit growth and identify agricultural practices that can improve fruit yield and quality. Moreover, it could be applied to schedule water deficit application in crops to control fruit quality.

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