Chapter 4
Process-Based Simulation Models Are Essential Tools for Virtual Profiling and Design of Ideotypes: Example of Fruit and Root

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Abstract Process-based simulation models (PBSMs) combine, in various mathematical frameworks, many biological functional hypotheses on responses of plant processes to environmental fluctuations. Model simulated responses can be analysed in the context of adapting the current agricultural systems to the changing environment. From loads of simulations made with various cultural practices, these models allow the virtual profiling of plants and a mere analysis of how processes interact when crops are perturbed by one or several changes. They allow also describing the development of plant traits as a consequence of environmental and genetic conditions. Such knowledge is required to decipher the genotype×environment×management (G×E×M) interactions so as to build genotypes adapted to particular conditions, i.e., plant ideotypes. Two PBSMs dealing with (1) fruit quality and sensitivity to diseases and (2) root system architecture, respectively, are shortly described in this chapter. These models have been used to analyse various fruit and root properties, to deconvolute G×E×M interactions and to identify ecophysiological traits related to crop yield improvement, root foraging performance and fruit quality. PBSMs appear to be powerful tools to phenotype plants at the process level in a comprehensive and "costless" way.

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4.1 Introduction

Plant production systems evolve regularly so as to cope with a growing worldwide demand of food production and important issues of quality, plant health, food safety and respect for the environment. Moreover, this adaptation must encompass the global climate change context. As a consequence, the most critical question for our future could be how to design the best possible combinations of genetic resources and environmental conditions (or cultural practices) able to achieve such objectives.

A prerequisite for performing such a design concerns the analysis of genotypic and environmental effects on plant physiological traits governing development and functioning and also on traits related to plant production, product quality, and plant health. Many plant scientists put the focus on genotype studies using profiling technologies. These techniques probe many genes, transcripts, proteins or metabolites, at once. The profiling technologies allow the analysis of system-wide responses (Hennig 2007; Kopka et al. 2004), which will undoubtedly help to understand 'how things work' at the cellular level. However, it is not always easy to interpret clearly the observed '*omic*' changes in terms of plant functioning because gene functions may be known at the cellular level but they are rarely identified in terms of plant responses while our knowledge of gene regulation in relation to the environment remains weak. In addition to these studies, one way to improve our understanding of plant responses is to use process-based simulation models (PBSMs). From the making of a load of simulation runs under various environmental conditions and/or cultural practices, it is possible to use these models to perform a virtual profiling (see below) and a mere analysis of how the plant system works, i.e.*,* how the numerous processes interact when the plant is perturbed by one or several changes at the cellular level (Génard et al. 2010). Indeed, PBSMs offer a theory describing how the system components causally interact to produce a given outcome. From this viewpoint, Peck (2004) regards simulation as "the creation of a possible world that is constructed *in silico* using computer programs to represent the processes under consideration".

In line with this idea, some agronomists and geneticists have proposed a smart approach that consists of analysing variation in the development of plant traits via PBSMs as a consequence of environmental and genetic factors (Hammer et al. 2005; Yin et al. 2005). Such PBSMs allow to virtually analyse how plants react to changing environments, cultural practices and genetic variability. The main expectation of such an approach is to decipher genotype×environment×management (G×E×M) interactions (Asseng et al. 2002; Boote et al. 2003; Chapman et al. 2003; de Dorlodot et al. 2007) to build genotypes adapted to particular conditions, e.g.*,* critical pedoclimatic situation, innovative crop management, future climates (Hammer et al. 2002). Thus, in contrast with the strategy developed in the past, researchers do not seek any longer genotypes adapted to all conditions but particular ones. Indeed, specifically adapted genotypes perform relatively better than other ones under a set

of conditions of particular interest, or lead to better environment-friendly production systems. Progress in this direction will clearly depend on the genetic information available on these related processes that will be injected in the PBSMs. This specific integration is the subject discussed in Chap. 1 of this book by Baldazzi et al.

New 'ideotypes' are real or virtual plant cultivars expressing an ideal phenotype adapted to target environments (Letort et al. 2008; Tardieu 2003). To design ideotypes, PBSMs are viewed as essential tools. Indeed, within a target environment, they can simulate a large number of virtual genotypes, each one being characterized by a set of genetic parameters. The challenge is now to identify a few of these 'ideotypes' among a myriad of simulated genotypes. The first attempts were conducted using techniques such as trial and error methods (Haverkort and Grashoff 2004; Herndl et al. 2007) and were quickly confronted with the difficulty and the hardness of the task. This is especially the case when multi-objectives are targeted. Indeed, the design of innovative cultivars is based on strong nonlinear antagonistic criteria with respect to influential constraints of production or environment. Resolving such problems is difficult using classical methods and it is known to be a matter of nonlinear multi-objective optimisation. Thanks to collaborations between biologists and mathematicians, effective methods have been proposed recently. They emerged from the field of multi-objective optimisation algorithms, e.g., genetic (Letort et al. 2008) and particle swarm (Qi et al. 2010) algorithms.

Two PBSMs dealing with (1) fruit quality and sensitivity to diseases and (2) root system architecture, respectively, will be shortly described in this chapter and used to analyse various fruit and root properties, sometimes including what Trewavas (2003) called *memory* and *compensatory* effects. The strength of PBSMs for analysing crop systems and for performing virtual profiling will be illustrated. We will also show how the use of model-based sensitivity analysis serves the selection of genetic traits necessary to design ideotypes. Finally, an approach for designing ideotypes will be described using the "fruit quality and sensitivity to diseases" PBSM.

4.2 What Are Process-Based Simulation Models for Fruit and Root Systems?

Process-based simulation models are collections of hypotheses and rules about the interrelationships linking processes to environmental variations and producing responses that can be analysed. The results are generally produced in the time domain and at an appropriate time step of the studied processes. They provide, therefore, a basis for the understanding of developmental, physiological and genetic phenomena, by dissecting complex traits into "elementary" processes. The classical notion of a single limiting factor is replaced by the idea of a sequence and/or network of different limitations operating through the plant's lifecycle. These interconnections and feedback regulations among the system components generate

unexpected global system properties, called emergent properties, which do not appear when the subsystems are individually considered (Trewavas 2006). Genotype×Environment (G×E) interactions are emergent properties of the whole system in which several processes interact. However, these interactions can also operate at the process level.

PBSMs permit the quantification of plant or organ responses to genetic, environmental, and management factors within a mathematical framework in which parameters are genotype-specific, thus allowing dynamic simulations of biophysical and physiological processes. They have yet been successfully used to deconvolute G×E interactions and to identify ecophysiological traits in studies designed to improve crop yield (Yin et al. 2000), root foraging performance (Pagès 2011), phenological development (Stewart et al. 2003; Welch et al. 2005), leaf elongation rate (Reymond et al. 2003) and fruit quality (Quilot et al. 2005).

Fruit quality traits have been seldom subjected to modelling, probably because they result from a poorly understood chain of processes encompassing only partly known steps of the complex underlying mechanisms (Struik et al. 2005). Conversely, root system architecture has been modelled for the last two decades (Dunbabin et al. 2013) but the models are generally too complex to give rise to ideotype conception (Pagès et al. 2004). PBSMs focusing on fruit size and composition have been proposed recently (Génard et al. 2007). They have proven their ability to produce emergent properties, i.e., to handle perturbations to any process and self-correct them as real plants do. The structure and some properties of these models will be presented hereafter.

4.2.1 Virtual Fruit Model

The first attempt towards integration and simulation of multiple quality traits has been made in the Virtual Peach Fruit model (Lescourret and Génard 2005). This PBSM (Fig. 4.1a) integrates many sub-models dealing with fruit growth and quality elaboration, and had its genesis in a model proposed by Lescourret and Génard (2005) including three existing process-based sub-models describing dry mass, sugar and water accumulation in the fruit flesh. Then, supplementary sub-models were added to describe skin conductance and microcracking (Gibert et al. 2005), respiration and citric acid accumulation (Lobit et al. 2003; Wu et al. 2007), and ethylene emission (Génard and Gouble 2005).

For now, the Virtual Peach fruit model describes the carbon (C) balance of a fruit-bearing stem. The available daily pool of C assimilates builds up from leaf assimilation plus possible C mobilized from the reserves. Carbon is allocated according to organ demands and priority rules. The fruit flesh is assumed to behave as one big cell. The carbon flow entering the fruit is partitioned into several compounds: four sugars (sucrose, sorbitol, glucose and fructose), other fruit compounds globally considered and the respired CO_2. Water flows into the fruit following the
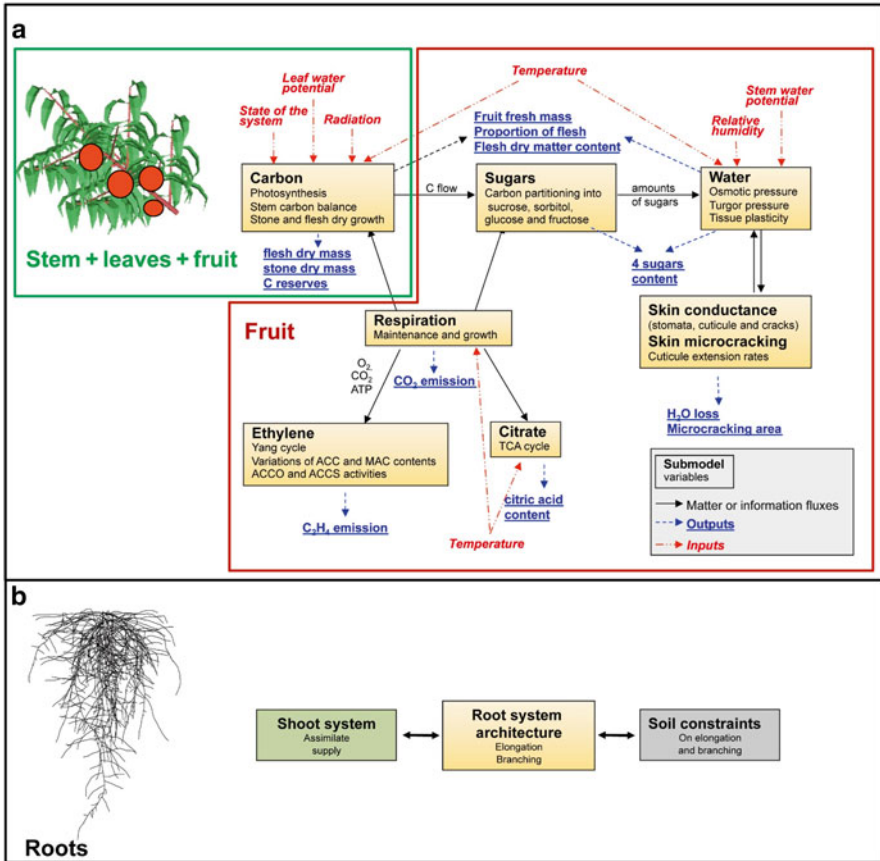
Fig. 4.1 Virtual fruit and root system architecture models. (**a**) Schematic representation of the relationships between sub-models as considered in the Virtual Fruit model. The sub-models simulate carbon balance of a fruit bearing stem, sugars and citric acid metabolism within the fruit, fruit water balance, skin conductance and microcracking, fruit respiration and ethylene metabolism. The inputs of the model are weather data, leaf and stem water potential, and the number of leafy shoots and fruits on the stem. The outputs (*underlined*) are flesh and stone masses, sugar and acid contents, skin microcracking and emission of gases. (**b**) The root system architecture model combines two main developmental processes (root elongation and branching) which are described by a number of rules with parameters. These processes are also affected by the assimilate supply (from the shoot system) and by the soil constraints

differences of hydrostatic and osmotic pressures between the xylem or phloem and the fruit. Changes in the fruit volume are predicted by the Lockhart equation as a function of turgor pressure. Fruit transpiration is calculated from the overall skin conductance to water vapour, by adding the individual conductance of stomata, cuticle and cracks. Cracks are assumed to happen when the pulp expansion rate exceeds that of the cuticle. The rate of citrate metabolism is calculated as the product

of a 'synthesis potential' by an 'efficiency level', which depends on respiration intensity. The ethylene sub-model simulates its biosynthetic pathway as a function of ATP supply, O_2 and CO_2 tissue concentrations, and its regulation by 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase. The inputs of this PBSM are weather data (i.e., global radiation, temperature and air relative humidity), leaf and stem water potential, and the number of leafy shoots and fruits on the stem. The parameters of the model have been estimated for the peach species in the framework of a large research programme undertaken over the last 10 years.

The Virtual Peach Fruit model has allowed the simulation of complex behaviours of fruit growth and quality traits in response to environmental fluctuations (Fig. 4.2). For example, compared with optimal water supply (W), the response to the alternation of periods of restriction and normal irrigation (S/W) shows a "compensatory growth" phenomenon, observable after re-watering when fruits regain that bit of growth lost during the period of stress. The model simulations show also clearly that fruits facing continuous water stress (S) maintain their growth rate, while fruits becoming water-stressed after a period of normal watering (W/S) experience a sharp slowdown in growth. This implies that S fruits adapted to drought but W/S fruits did not. In the model, these growth patterns are related to sugar concentration changes, the sustained fruit growth being linked with high sugar concentrations under conditions of water deficit. In real plants, Trewavas (2004) called this kind of adaptation a 'memory effect'. He reckons that compensatory growth is a corrective mechanism involving a feedback control to achieve a developmental goal (Trewavas 2003). We can hypothesize that the Virtual Peach fruit model mimics such a "sugar signal"-based mechanism, if we assume that the increase in sugar concentration during the stress period (as shown in Fig. 4.2) promotes growth after re-watering.

These responses are not accounted for by any of the Virtual Fruit sub-models taken separately, but they result from feedback regulations, which emerge from the entire Virtual Fruit model. This illustrates the usefulness of PBSM to analyse complex responses of quality traits to environmental variations.
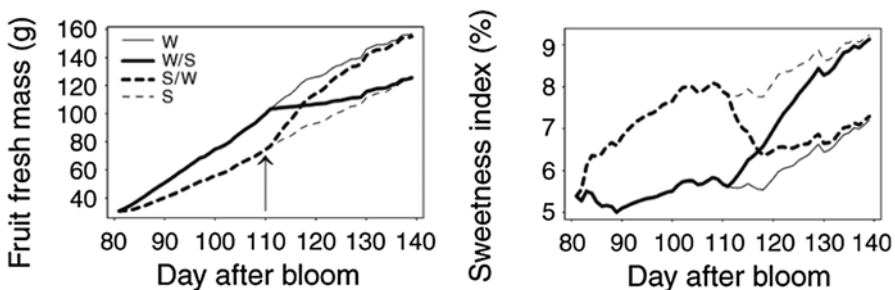

Fig. 4.2 Time course of fruit fresh mass and sweetness (correlated to sugar concentration) simulated by the Virtual Peach Fruit model under four scenarios of water conditions (W = normal water condition, W/S = normal then stressed water condition, S/W = stressed then normal water condition, S = water stress). The *arrow* indicates the time when the water condition changed

4.2.2 Root System Architecture Model

Pagès (2011) presented the main aspects of a model of root system architecture (RSA) to link elementary developmental processes (and associated traits) at the individual root level to complex (or integrated) traits describing foraging performance at the whole root system level. This model was then calibrated and evaluated on a panel of different species belonging to various plant families (Pagès et al. 2014). It is a discrete model simulating the 3D RSA with a 1-day time step. The dynamic virtual root system is represented as a set of small segments with different attributes (location, diameter, age, connection). Basically, the model includes three types of interacting processes: (*i*) a number of morphogenetic rules define the elongation and branching of individual roots; (*ii*) the overall demand of roots for growth is compared with the biomass allocation to the root system within the whole plant and can be reduced in the case of limitation; (*iii*) each root meristem (located in the soil space) experiences local conditions, which can reduce its potential elongation and branching (e.g., strong soil or fresh temperatures).

Regarding the morphogenetic rules governing the RSA dynamics, it is noticeable that the size of the root meristems plays a central role. Potential elongation rates of individual roots are linearly linked to their tip diameter, the slope of this relationship being assumed to be a genetic parameter. For each species, the elongating-roots' tip diameter ranges between the minimal and maximal values that are both specified as genetic parameters. The branching process defines both the longitudinal spacing of successive lateral roots on their mother, and the tip diameter distribution among lateral roots. On average, their diameter is linearly linked to that of their mother root and it is supposed to be variable, the coefficient of variation being also a genetic parameter.

This very simple model (ArchiSimple) benefited from a long-term experience on RSA modelling (Pagès and Ariès 1988), which facilitated the necessary simplification (Fig. 4.1b). However, it was designed to include ecophysiological concepts such as sink strength (through the meristem size), possible carbon source limitations and the effects of some soil characteristics to modulate root elongation (e.g., soil strength or soil temperature). These processes are known to exhibit strong genetic variations (de Dorlodot et al. 2007).

From the virtual RSA, it was possible to estimate a foraging performance (Pagès 2011), considering that root systems take up soil nutrients located around the roots, within a given rhizosphere volume characterised by its distance to the roots. This distance reflects in fact the type of nutrient that is targeted. For non-mobile ions (e.g., phosphate) this distance is only a few millimetres whereas for mobile ions (e.g., nitrate) or water it attains several centimetres. It is worth noticing that an integrated trait such as the colonised root volume can be calculated on virtual 3D root systems, but it is actually impossible to assess on real root systems.

Thus, in this case, the model, that can be used to design root ideotypes, includes in fact an RSA model explicitly connected to the soil and the shoot system and a very simplified uptake model representing the mere limiting soil resources (water and/or nutrients).

4.3   Virtual Profiling on Roots and Fruits

Our purpose is to show how PBSMs can be used to perform virtual profiling to phenotype plants in terms of ecophysiological processes. The following examples describe two possible, but not unique, approaches. On the root systems, our profiling proceeded from the analysis of a few parameters studied on a large virtual root population while on fruits, it came from the study of a large set of variables on two contrasted genotypes.

4.3.1   Roots

Using the ArchiSimple model, a large number of root systems were simulated and used to explore the relationships between their overall performance and their elementary developmental traits.

For this purpose, each parameter was given random values in its plausible interval, defined from the literature. The experimental design conformed to a paving, as suggested by Saltelli et al. (2008). Root foraging efficiency was defined as the ratio of the total rhizospheric volume to a theoretical volume without any root overlap, i.e., with no redundant soil exploration. From this in silico experimentation several main results were obtained, which illustrates the interest of such approaches. The overall foraging efficiency was highly dependent on the overall size of the root system, because the rhizosphere of different roots tended to overlap more and more during root system development and extension (Fig. 4.3). As expected, this overlap was dependent on the rhizosphere size (distance). The approach allowed quantifying this important phenomenon, which is usually neglected, even though root systems are particular objects in which roots are aggregated in space, mainly because they are connected as a branching system. The efficiency varied considerably from one root system to another, even for a same total root length, and the variations were highly dependent on some parameters. Favourable ranges were defined for particular parameters, such as the inter-branch distance. Moreover, the overall tip-diameter range allowed by the virtual phenotype (called heterorhizy) was shown favouring efficiency. Interestingly, the genetic parameter modulating the variation among lateral root diameters had also a large impact on the overall efficiency. When a sub-population of efficient root systems was selected (elite population), it was shown that some parameter associations were excluded and conversely other associations between parameter values were favoured. Thus, correlations between parameters enabled to quantify the trade-offs in the elite population.

Thus overall, the use of such a PBSM confirmed its value to decipher these very complex relationships and allowed to bridge two scales: one on which developmental processes can be studied, and the other on which agronomic performances can be evaluated.
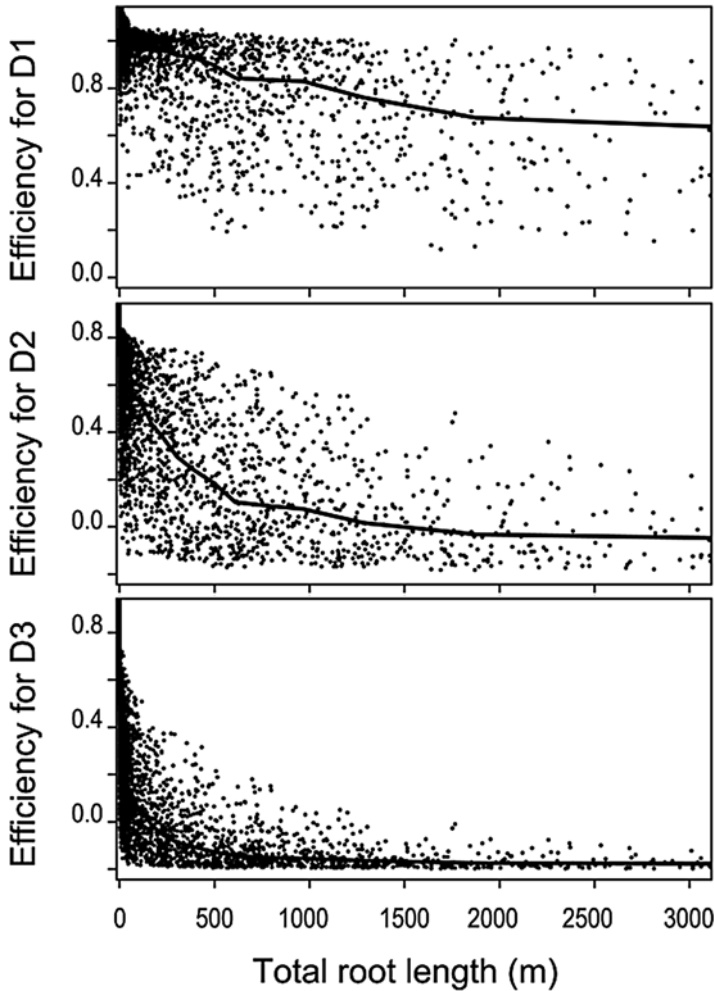
Fig. 4.3 Relationship between foraging efficiency (quantifying the rhizosphere overlap between different roots) and size of the root system (redrawn from Pagès (2011)). Efficiency varies between 0 (full overlap) and 1 (no overlap). The considered rhizosphere radius was 3 mm (D1), 10 mm (D2) or 30 mm (D3). Each point represents a root system simulated by the model of Pagès (2011) on which the total length and the rhizosphere characteristics have been calculated. The *lines* represent the trends, calculated with the "loess" function in the R software

4.3.2 Fruit

The 'Virtual Fruit' model has been used to simulate wild type and mutant plants over a period of 70 days for fruit growth. To simulate the mutation in the model, we decreased by 70 % the value of the unique parameter that modulates the fruit sugar uptake. We used the model to calculate the daily value of 39 variables related to

various processes (Table 4.1). The considered functional variables were in most cases rates such as photosynthetic rate (g CO_2 m^{-2} d^{-1}) or relative rates such as daily variation of sucrose content (d^{-1}). Some physical characteristics such as turgor pressure or conductance were also considered since they are proportional to relative growth rate or fluxes.

By analogy with some current presentation of 'omic' results, we produced a heat map of the 39 variables (Fig. 4.4), which appears as a virtual profile fingerprint summarizing the processes impacted by fruit ageing. The wild-type genotype showed very contrasted temporal variation patterns according to the variables. Indeed, the relative variation of ATP, glucose and fructose suffered large temporal fluctuation mainly due to changes in the climatic environment. Other variables such as fruit turgor pressure showed slow oscillations during fruit development. Most of the variables followed a temporal gradient with three distinct periods. Early in the season there was a high production of citric acid in the fruit. During the mid-season we detected high activities of stone growth and sucrose accumulation (SU). In the late period of fruit maturation, ethylene production increased.

For the mutant plants, the general pattern was similar to that observed for the wild-type genotype. The same variables were involved in three main growth periods and oscillatory behaviours were also simulated (Fig. 4.4). During the first period, the variables involved remained almost quantitatively unchanged. By contrast, changes appeared in the two last periods since the mutation triggered a large effect in several leaf and fruit variables (photosynthesis, growth, respiration and metabolism) and delayed the fruit developmental rate. All variables involved in the mid-season period had lower intensities compared with the wild-type genotype. Similarly, most variables involved in the fruit maturation period showed lower activities, except enzymes involved in the sucrose and sorbitol metabolisms (Eso and Esu), which maintained similar activities. The flesh osmotic potential (Os) was the unique oscillatory variable showing higher values in the mutant.

At the end, the mutation involved in the fruit sugar uptake had a strong effect in most fruit processes and on the plant source activity throughout fruit growth. The single mutation perturbed the whole system and impacted fruit quality, decreasing fruit mass and skin cracking and lowering ethylene emission (Fig. 4.5). Similar results were also obtained with a change in fruit load, which impacted also most of the plant and fruit processes (Génard et al. 2010).

Such a virtual profiling approach could lead to new ways of exploring *in silico* the impact of mutations or naturally occurring genetic variations. A thrilling challenge for the future will be to connect virtual and 'omic' profilings. Two main approaches are possible, one being empirical and the other mechanistic. A means to facilitate virtual and 'omic' profiling connection is to perform 'omic' and virtual profiling on the same subjects and to use data-mining technologies such as the bidirectional orthogonal projection to latent structures (O2-PLS) method recently proposed by Bylesjö et al. (2007) to search for links between them. To go further, the mechanistic integration of information generated by 'omics' technologies into

Table 4.1 List of the processes and functional variables of the Virtual Fruit model considered for the virtual profiling. Label, unit and range of variation are given for each variable

Organ	Process	Variable	Label	Unit	Range of values (in Fig. 4.4)
Stem and Shoots	Carbon balance	Relative stem C-storage rate	Cst	d^{-1}	−0.0022 to 0
		Relative shoot growth rate	Sh	d^{-1}	0 to 0.1
		Relative shoot C-storage rate	Csh	d^{-1}	−0.014 to 0.009
		Relative stem and shoot maintenance respiration	MRs	d^{-1}	0.0009 to 0.0017
		Leaf photosynthetic rate	Lph	$g\ m^{-2}\ d^{-1}$	1.37 to 4.01
		Maximal leaf photosynthetic rate	Pm	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$	5.54 to 19.49
Fruit	Carbon balance	Relative fruit demand	Fde	d^{-1}	0.0012 to 0.0318
		Fruit photosynthetic rate	Fph	d^{-1}	0.004 to 0.187
		Relative flesh respiration rate	Fre	$\mu mol\ CO_2\ g^{-1}\ DM\ d^{-1}$	0.077 to 0.348
		Relative flesh dry mass growth rate	Fd	d^{-1}	0.0031 to 0.0459
		Relative stone dry mass growth rate	Sd	d^{-1}	0.0019 to 0.0244
	Water balance	Relative transpiration	Tr	d^{-1}	0.004 to 0.104
		Relative water influx	Flux	d^{-1}	0.07 to 0.36
		Flesh turgor pressure	P	bar	5.0 to 5.15
		Flesh osmotic potential	Os	bar	11.6 to 14.6
		Relative flesh fresh mass growth rate	Ff	d^{-1}	0.02 to 0.36
		Relative stone fresh mass growth rate	Sf	d^{-1}	0.0013 to 0.0226
	Skin	Crack conductance	Crco	$cm\ h^{-1}$	0.0 to 81.2
		Stomatal conductance	Sco	$cm\ h^{-1}$	11.5 to 27.7
		Cuticular conductance	Cuco	$cm\ h^{-1}$	59.8 to 357.1
		Skin area growth rate	Skin	$cm^2\ d^{-1}$	0.07 to 1.64

(continued)

Table 4.1 (continued)

| Organ	Process	Variable	Label	Unit	Range of values (in Fig. 4.4)
	Sugar and citric acid metabolism	Relative daily sucrose content variation	SU	d^{-1}	−0.0007 to 0.0055
		Relative daily sorbitol content variation	SO	d^{-1}	−0.0027 to 0.0039
		Relative daily glucose content variation	GL	d^{-1}	−0.0009 to 0.0022
		Relative daily fructose content variation	FR	d^{-1}	−0.0004 to 0.0022
		Metabolizing sucrose enzyme activity	Esu	d^{-1}	0.0008 to 0.041
		Metabolizing sorbitol enzyme activity	Eso	d^{-1}	0.32 to 0.89
		Activity of enzyme metabolizing glucose and fructose in other compounds	Eglfr1	d^{-1}	0.015 to 0.218
		Activity of enzyme metabolizing glucose and fructose for respiration	Eglfr2	d^{-1}	1.15 to 9.19
		Relative daily citrate content variation	CI	d^{-1}	−0.71 to 2.19
	Ethylene metabolism	Relative daily ACC content variation	ACC	$mol\ g^{-1}\ d^{-1}$	−0.008 to 0.009
		Relative daily MACC content variation	MACC	$mol\ g^{-1}\ d^{-1}$	−0.0008 to 0.0031
		Relative daily C_2H_4 content variation	C2H4	$mol\ g^{-1}\ d^{-1}$	−0.0003 to 0.0003
		Relative daily O_2 content variation	O2	$mol\ g^{-1}\ d^{-1}$	−0.051 to 0.075
		Relative daily CO_2 content variation	CO2	$mol\ g^{-1}\ d^{-1}$	−0.042 to 0.029
		Relative daily ATP content variation	ATP	$mol\ g^{-1}\ d^{-1}$	−0.029 to 0.022
		C_2H_4 emission	C2H4em	$\mu mol\ g^{-1}\ h^{-1}$	0 to 0.001
		ACCO activity	ACCo	$\mu mol\ g^{-1}\ h^{-1}$	0 to 0.0007
		ACCS activity	ACCs	$\mu mol\ g^{-1}\ h^{-1}$	0 to 0.0025
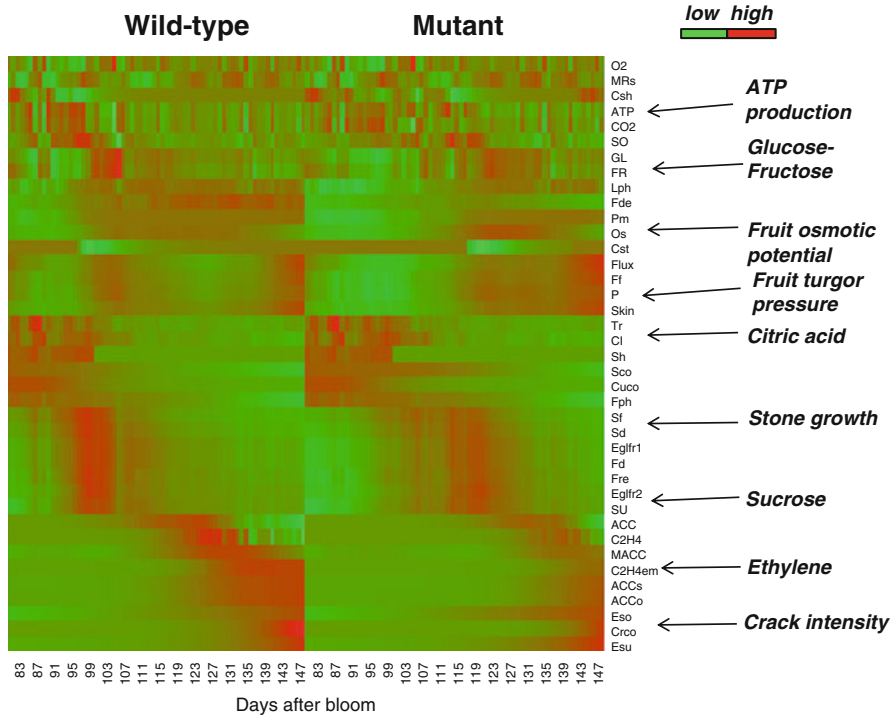
Fig. 4.4 Heat map surface of model variables during the fruit developmental period of the wild type and mutant genotypes. In the heat map, each row is a variable and each column is a day after full bloom. Abbreviations of the variables are defined in Table 4.1. The values increase from *green* to *red*

models could provide a global view of how plants operate. So far, upscaling attempts from gene to cell have been successfully undertaken (Tomita et al. 1999), but further upscaling towards the organ or the plant levels has not yet been performed (Baldazzi et al. 2012). A possible avenue would be to embed mechanistic metabolic models into PBSMs such as the Virtual Fruit model. For instance it could be possible to substitute its current simplified sugar model with a more detailed one, such as the sugar metabolism model developed by Uys et al. (2007) for sugarcane. The Virtual Fruit model would thus produce inputs for the metabolic model, which could in return simulate the production of metabolites along the metabolic pathways. By comparing simulations with real metabolic profiles obtained through metabolomic studies, it would be possible to test the benefit of inserting such metabolic model within the Virtual Fruit model.
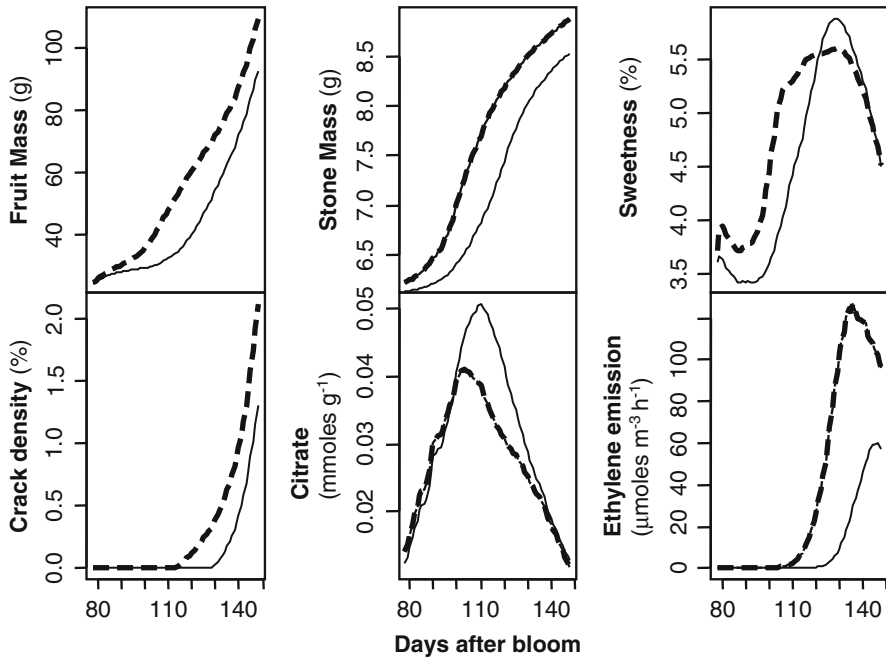
Fig. 4.5 Comparison of wild-type (*thick dashed lines*) and mutant (*thin lines*) simulation outputs generated by the Virtual Fruit model. Variation of fruit growth, sweetness (equivalent sucrose), citric acid content, cracking and ethylene production are presented during the fruit developmental period

4.4 Ideotype Design

4.4.1 Sensitivity Analysis, a Key-Step Before Designing Ideotypes

Sensitivity analysis (SA) is a statistical technique allowing to assess the impact of changing some input parameters on the model outputs (Blower and Dowlatabadi 1994). Saltelli et al. (2008) suggested conducting SA on the model (i) to test its accuracy, (ii) to prioritize the parameters before their estimation, (iii) to simplify the model and reduce its parameter number, and (iv) to identify the interactions between parameters. For example, ranking model parameters arises from sensitivity indices reflecting the main effect of each parameter and their interactions. Parameters having small or no effect on model outputs can thus be set to fixed values, leading to model simplification.

We can distinguish local SA methods from global ones. The former evaluate the impact of a very small variation around a given input value, while the latter study the output variability when that of the input covers the whole possible domain (Jacques 2011). Many SA methods have been used in the literature. These methods

have different bases, including elementary effects (Morris or *one-step-at-a-time* method), regression and correlation coefficients, variance decomposition (Sobol, FAST). Software and packages implementing these methods are largely accessible, for instance the *R sensitivity package*.[1] SA techniques are very useful to study the behaviour of complex numerical models such as ecophysiological models described in this chapter. Therefore, SA techniques were among the earliest tools used for the model-based design of ideotypes (Habekotté 1997). Indeed, SA methods coupled with process-driven biophysical models may help answering some '*what if*' questions before engaging in experiments (Fischer 1996). A SA of ecophysiological models under contrasted climatic conditions and/or agricultural practices allows identifying the most important parameters that mainly affect outputs of interest (i.e., targeted traits). For example, Quilot-Turion et al. (2012) performed an SA on the 'Virtual Fruit' model to identify the main parameters affecting fruit fresh mass, sweetness and crack density. The *'elementary effects'* screening method (Morris 1991; Saltelli et al. 1999) was used for this purpose. This method computes two sensitivity measures: the mean and the standard deviation of the distribution of the elementary effects associated with a given parameter. They respectively assess the overall influence of a given parameter on the output and on the interactions of a parameter with another one. In the study of Quilot-Turion et al. (2012), the sensitivity of each output variable to each parameter was quantified by considering a 10 % variation interval around each parameter reference value. For the 'Virtual Fruit' model, from about 60 parameters submitted to the SA, only few parameters had significant impact on the model outputs. We will show in the next section how these six genetic parameters can be combined to create interesting ideotypes.

Pagès et al. (2012) used a global SA on the root system architecture to link developmental parameters with the shape of the vertical root length distribution, which is often used to characterize the root system in crop models. This analysis allowed creating a meta-model that enabled model inversion to facilitate the estimation of developmental parameters from vertical root length density profiles (Pagès et al. 2012).

4.4.2 Multi-objective Optimisation Algorithms to Design Ideoypes

Designing environment-friendly production systems that produce safe food of good quality is an important challenge for the future. Indeed, such a production system may sustain the economic viability of farms. Today, one of the promising ways to tackle this issue is to identify the best combinations of genetic resources and cultural practices adapted to target environments. The idea here is to take advantage of the strong genotype×environment×management (G×E×M) interactions in

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[1] http://cran.r-project.org/web/packages/sensitivity/index.html

order to design plant ideotypes that meet these objectives. Such an approach relies on two main pillars: the potentialities offered by integrating genetic information into process-based models and the merger of breeders and agronomists (Messina et al. 2009). Therefore, model-based design of ideotypes aims at finding the most suitable combinations of genetic parameters, genotype fingerprints and cultural practices adapted to target environments (Letort et al. 2008; Tardieu 2003).

It should be clearly stressed here that model-based ideotype design involves many conflicting criteria subject to hard constraints and therefore, it shows a number of difficulties. Firstly, there is no explicit mathematical relationship linking the decision variables (genetic parameters and management practices) to the models' outputs (criteria and constraints). Secondly, these relationships are probably nonlinear as suggested by the results of simulation techniques. Thirdly, it is impossible to test all the candidate combinations due to their huge number. Fourthly, the fitness landscapes to be explored are usually complex and have many local optima (Messina et al. 2009). Therefore, model-based ideotype-design triggers a very difficult optimisation problem. In order to proceed, we need efficient optimisation algorithms able to tackle these difficulties that resist classical optimisation and simulation methods. In a large number of domains, nature-inspired optimisation algorithms (e.g., genetic algorithms, ant colonies, particle swarm optimisation algorithms) appear efficient to solve difficult optimisation problems. These algorithms allow exploring highly dimensional solution spaces in a reasonable computation time. In addition, they do not require any derivative information, and can address the complex multi-objective optimisation problems (e.g., very large search spaces, uncertainty, noise, disjoint Pareto curves). As a result, the use of nature-inspired algorithms has recently expanded in the domain of model-based ideotype design (He et al. 2012; Kadrani et al. 2012; Letort et al. 2008; Qi et al. 2010; Quilot-Turion et al. 2012) and optimisation of management scenarios (Grechi et al. 2012). These methods provide a set of diversified solutions to decision-makers and let them have the final choice of the best-suited trade-off between criteria.

We will illustrate now the use of nature-inspired multi-objective optimisation algorithms to design ideotypes. Our illustration concerns the peach fruit susceptibility to a pathogenic fungus (*Monilinia* spp.), responsible of the peach brown rot. This infection is largely occurring through fruit-wounds and it is proven that cuticular cracks play a major role for fungal infection (Gibert et al. 2009). Cuticular crack density has been shown to rise with fruit growth rate, which in its turn varies with management practices such as irrigation regimes and fruit thinning (Gibert et al. 2010). The cracks represent large opportunities for fungal infection and contribute also to fruit water losses by transpiration, influencing thereby fruit growth and quality build-up. *Monilinia* spp. causes in France a rising problem mainly due to the current reduction of fungicide usage in orchards for ecological, economic and human-health reasons. This short description illustrates clearly the conflicting objectives of the peach growers since the production of big fruits, which are generally the sweetest in taste, leads inevitably to elevated *Monilinia* attacks. In this case, growers should be interested to find an adequate trade-off between quality and fruit health performances. It is needless to say that this trade-off depends also on the

environmental conditions. Thus, overall we must search for the best possible exploitation of G×E×M interactions, seeking specific adaptation of some genotypes to target population of environments (TPE) (Cooper and Byth 1996). The idea is to cluster crop environments according to their limiting factors and the frequency of their occurrence. Within a class of TPE, a given genotype may have an expected and stable behaviour. Thus, the optimisation step must be performed in various TPE differing by cultural practices and/or climate profiles so that different ideotypes can emerge. Therefore, the definition of relevant target environments in which the limiting factors are well identified, represents a major step to focus the subsequent ideotype design.

As an attempt to sort out this problem, we used the "Virtual Fruit" model to design peach ideotypes with large fruit mass, good sweetness, and low density of skin cracks, i.e., low sensitivity to brown rot.

As mentioned before, the SA performed on the model identified the main parameters affecting some important outputs of the Virtual Fruit model, i.e., fruit mass, sweetness, crack density. From this SA, we selected six parameters affecting the processes of vegetative activity, i.e., fruit growth, sugar metabolism, stone-pulp partition, transpiration and water fluxes. These parameters are considered to be genotype-dependent and mostly independent of the environment. Consequently, each genotype may be considered hereafter as a set of six genetic parameters. We ran the model under two contrasted modalities of irrigation regimes and thinning intensities in order to analyse the impact of cultural practices on the optimised solutions. Irrigation and thinning practices are particularly relevant in the peach system due to their ecological impact and labour requirement. Moreover, both have large effects on fruit yield, quality and cuticular cracking. The optimised solutions emerged from the coupling of the model with different nature-inspired multi-objective optimisation algorithms (for details, see Kadrani et al. 2012, 2013; Ould-Sidi et al. 2012; Quilot-Turion et al. 2012). All algorithms provided a large diversity of solutions, among which it was possible to choose the best-suited trade-off between criteria according to a particular objective. Our results confirmed that the solution domain was strongly constrained by the fruit mass and the microcrack characteristics and that there was a strong antagonism between the criteria considered (Fig. 4.6). For example, we identified solutions matching current breeding schemes where fruit mass is the sole rated criterion. However, we also identified some interesting optimised solutions representing a breakthrough in the current schemes, which could be considered in the future as they do favour organoleptic quality and environment-friendly practices. The global impact of crop load appeared high on fruit mass and crack density and moderate on sweetness. Irrigation regime displayed a small impact on the three criteria in case of low crop load but gave rise to contrasting phenotypes in case of high crop load. We found that whatever the scenario, ideotypes with lower fruit mass exhibited greater sweetness and had lower crack density on their skin. According to the 'Virtual Fruit' model, we may predict that it is not possible to get a set of six genetic parameters leading to ideotypes of very high fruit mass and sweetness but low sensitivity to brown rot. The figure reveals that commercial standards picking out cultivars with big fruits promote high
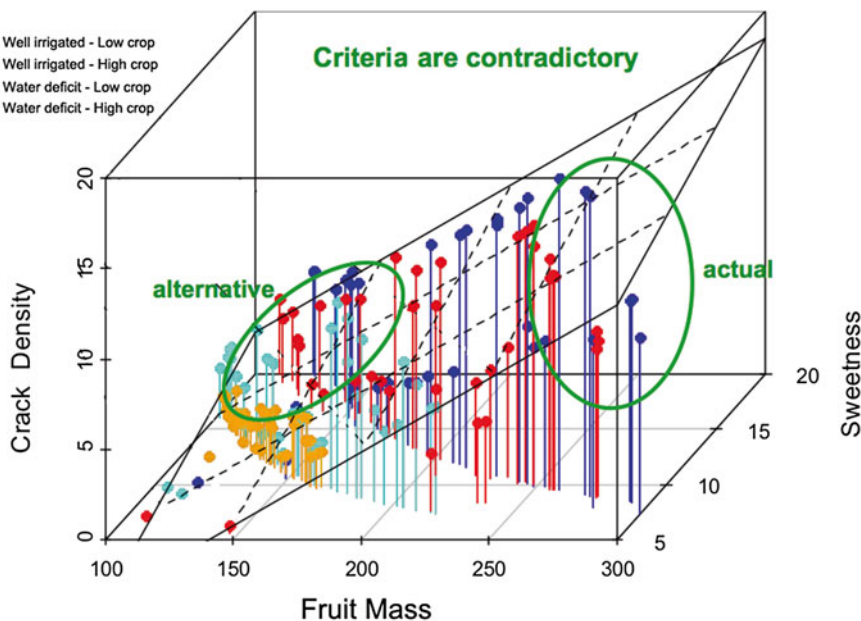
Fig. 4.6 Virtual Fruit outputs for the set of final solutions for four environmental scenarios (two levels of crop load and two water regimes). The optimization was done using the NSGA-II (Non-dominated Sorting Genetic Algorithm II) algorithm, which explores complex search spaces in a reasonable computation time. More details can be found in Quilot-Turion et al. (2012). Cuticular crack density is plotted against fruit mass and sweetness. The highlighted solutions (green ovals) identify the actual breeding strategies versus putative alternative strategies devising a compromise between the three targeted traits

sensitivity to brown rot and low fruit sweetness. According to our simulations an interesting compromise would be to breed for cultivars with lower fruit mass, obtained for example by decreasing the hydraulic conductance of tissues (one of the six parameters tested) in order to get sweet fruits with low sensitivity to brown rot. This would be an acceptable trade-off favouring organoleptic quality and environmentally friendly practices.

The results of these pioneer studies illustrate the value of the multi-objective optimisation approach. The next important step will be applying this approach to a prospective search of ideotypes adapted to future climates or to seek opportunities for crop expansions into new areas. This is particularly relevant in the case of perennial crops that are settled in an orchard for decades. Historical data or outputs from climate models could be used as inputs in the Virtual Fruit model. The output simulations could thus help identify the main traits of genotype adaptation to changing climates for the future.

However, an important initial step is needed before using this approach to such a prospect. Indeed, a main weakness of the methodology is the current lack of quantitative relationships between genes and model parameters. In fact, this approach

simply provides a picture of the optimised space of solutions from the viewpoint of the system functioning under the constraint of biophysical limiting factors. Presently the suggested solutions represent ideal genotypes that breeders may not be able to create. In order to produce more realistic genotypes, genetic constraints (such as pleiotropic and epistatic effects, and G×E interactions; see Chap. 1 of this book by Baldazzi et al.) must be integrated into the optimisation scheme.

4.5 Conclusion

To breed for cultivars that can adapt to changing climates to sustain or to increase yield and product quality, we need a better understanding of the complex interactions among the numerous factors that drive resource acquisition, distribution and storage in response to environmental stimuli. Moreover, there is an urgent need to identify and track key genes involved in plant adaption to stress-prone environments. The PBSMs described in this chapter have proven very efficient for unravelling plant complexity and plasticity in response to environmental stimuli or genetic perturbations. In addition, we have illustrated how PBSMs can be used as powerful tools for phenotyping plants at the process level in a comprehensive and "costless" way. As a consequence, these models should also be regarded as high-throughput phenotyping platforms that may complement the more expensive genetic, proteomic or metabolomic platforms. Indeed our assessment of the Virtual Fruit model and the root system architecture model shows clearly that PBSMs may help us to disentangle the links between genotypes (i.e. a set of parameters) and phenotypes. On the one hand, we can identify optimized sets of genetic parameters to achieve a target phenotype. On the other hand, models help us understand how a given phenotype may emerge from a specific genotype. Over the last decade, near-isogenic lines, mutants, transgenic and mapping populations have been developed for several traits related to fruits quality. Such materials give scientists the opportunity to evaluate the hypotheses introduced in the models and to highlight important regulation loops.

Nevertheless, we are still far from the rise of new cultivars based on model-designed ideotypes. One main reason is the gap between model parameters and genes or physiological functions. To progress in this field, more work is expected in order to fill the gap between genetic information concerning the traits and processes included in models. Moreover, PBSMs must be refined by more mechanistic details able to enlarge their ability to simulate the complexity of plant and organ functioning. Adding genetic and genomic information on gene actions and interactions into PBSMs will help modellers to unravel and strengthen physiological assumptions and equations in their models. It will also help to reduce some uncertainty about the genetic knowledge caused by environment interactions. In our opinion, it is not essential to understand fully a trait genetics and physiology to model it at an operational level. Nevertheless, modellers are seeking adequate data sets desperately, offering time series and accurate description and characterisation of cultural conditions and genotypes, so as to parameterise and evaluate their PBSMs. In this

prospect, it would be advisable to establish a large database including phenotype data measured at plant, organ and process levels and cross this information with molecular and genetic databases.

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