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SOURCE-SINK RELATIONSHIPS IN BLUEBERRY (*Vaccinium corymbosum* L.) PLANTS: FRUIT AND LEAF RESPONSES AND A SIMULATION MODEL OF FRUIT GROWTH AND SUGAR CONCENTRATION

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SOURCE-SINK RELATIONSHIPS IN BLUEBERRY (*Vaccinium corymbosum* L.) PLANTS: FRUIT AND LEAF RESPONSES AND A SIMULATION MODEL OF FRUIT GROWTH AND SUGAR CONCENTRATION

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…dedicada a mi Madre, Paula e Ismael

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Summary and thesis outline

The yield and quality of harvested organ in plants well supplied with water and inorganic nutrients depends primarily on i) the accomplishment of photosynthesis and ii) the transport of carbon compounds from source of assimilates to heterotrophic cells which constitute metabolic sinks. The complex interplay of availability of supply and demand of carbon (C) between source (mainly leaves) and sink organs (mainly harvested organ) has been historically studied in terms of source-sink relationships (SSR). Unbalances in SSR occur when the C source offer fails to match the demand of the C sinks or when C assimilation by the source organs is down-regulated by low C demand by sinks. In either case, the plants annual outcome is affected, which has implications on yield, quality of harvested organ and biomass gain to support the following production cycle.

Unlike other fruit crops, research based on source-sink approach has been scarcely carried out in blueberry plants (*Vaccinium corymbosum* L.) despite that SSR of this crop is annually modified via winter pruning (dormant pruning). This practice regulates fruit load and plant architecture that permits sunlight penetration and interception by leaves for driving carbon assimilation to sink organs.

In this thesis, it was investigated the effect of SSR on vegetative and productive responses, fruit quality and leaf traits of blueberry cultivar 'Brigitta'. Two work scales were used to study the effect of varying SSR: i) whole-plant, by manipulating of pruning intensity; since the shrub is the key level in which most variations in plant performance occurs and it is the target of most technical interventions, and ii) fruiting shoots, which is a unit where source-sink ratios can be easily achieved by removing leaves, fruits or both. Because, the use of individual shoots as the unit level for SSR studies requires isolation from the parental plant in order to avoid the buffer capacity of the rest of the plant, girdling was applied at this level. The corresponding and other available knowledge is taken to be integrated in an ecophysiological model (process-based model), which predicts growth and sugar accumulation of blueberry fruit.

The outline of this thesis begins with a general introduction. In Chapter I, we address the general hypothesis and objectives of this thesis. In Chapter II, a literature review was made, which aimed to define the source-sink concept, to describe the main factors regulating carbon supply and demand in plants, and to describe how source-sink relationships are approached by modelling.

In Chapter III, pruning intensity on 'Brigitta' blueberry plants was tested. We found that lesser removed wood stimulated a greater yield and berries per plant but reduced whole-canopy leaf area. This concomitantly resulted in different fruit loads as the ratio between fruits and canopy leaf area per plant changed. Pruning treatments led to varying berry weight, dry matter and soluble sugars (total and measured fractions) in fruits. These fruit quality traits were significantly correlated to fruit load through non-linear regressions. On the other hand, we found that fruit growth was source-limited early in the cell division and cell enlargement fruit growth phases, as relative fruit growth rate decreased. In this Chapter, photosynthetic light-response curves were constructed on sun leaves from fruit-bearing shoots similar in vigour, length and number of fruit per leaf (to avoid the effect of nearby fruits on the leaf photosynthesis) to test the hypothesis that light-saturated assimilation rate is increased when a high fruit load results from slight pruning. From light-response curves a mathematical model was fitted to estimate light-saturated photosynthesis (A^{sat}), dark respiration rate (R_d) and the apparent quantum use efficiency (a_{light}). The hypothesis was supported, indicating that photosynthetic capacity of blueberry leaves is increased by a high fruit load resulting from slight pruning. Decreased stomatic conductance resulted in lower leaf internal CO_2 concentration in leaves, indicating that a limiting CO_2 concentration in the stomata could be associated with the loss of CO_2 fixation capacity when fruit load steadily decreased. The R_d and a_{light} were unaffected, showing that changes in R_d were uncoupled with changes in A^{sat} and that the utilization of excitation energy was matched by a similar carbon metabolism rate when low light intensities is experienced by leaves, respectively.

In Chapter IV, we focused on the effect of SSR at fruit-bearing shoot level on physiological and structural leaf traits. Thus, A^{sat}, R_d, stomatic conductance (g_s), intrinsic water use efficiency

($WUE_i = A^{sat} / g_s$), soluble sugars, nitrogen (N), carbon (C) and photosynthetic pigments of leaves were evaluated in the course of the day. Treatments of SSR were adjusted by mean of removal either fruit or leaves or both. Girdling was applied in these shoots and additional non-girdled shoots were used as control of girdling. The data was analysed as function of treatments and the course of the day. A low sink demand induced by girdling and lower fruit load resulted in lower A^{sat}, g_s, N and total chlorophyll and in higher WUE_i, SSC, R_d, chlorophyll a/b ratio and carotenoids/total chlorophyll ratio (Car/Chl_{tot}). Variables other than A^{sat}, R_d, g_s, WUE_i and SSC were unaffected by the course of the day. The A^{sat} and g_s decreased during the course of the day, but A^{sat} decreased more than g_s in the afternoon increasing WUE_i. The SSC increased from morning to afternoon, whereas R_d picked at noon regardless treatments. For whole data set, A^{sat} was not limited by g_s and was closely and negatively correlated to SSC, indicating that sugar-sensing mechanisms had an important role on regulation of blueberry leaf photosynthesis under evaluated conditions. For treatments, N and A^{sat} were positively related, confirming the co-regulation between these variables. An enhanced Car/Chl_{tot} points towards a higher photo-protected state under lower sink demand. For measured variables, a matrix of correlation was made and results were discussed. From the study results, we suggested that changes in source-sink relationship at fruiting shoot level led to a rearrangement of physiological and structural leaf traits, which allows adjusting the daily balance between carbon assimilation and absorbed light energy.

In the Chapter V, an ecophysiological model (process-based model) was adapted to simulate fruit growth, sugar concentration and water relations and uptake of ‘Brigitta’ blueberry fruits as affected by fruit load resulted of different pruning severities. The model is based on the biophysical representation of water transport combined with the growth process stimulated by turgor and osmotic pressures of the fruit. The main state variable of the system are the amount of water in the fruit. The daily inputs of the model were fruit dry mass, temperature and relative humidity. The comprehensive theoretical model framework enabled us to predict the dynamic of blueberry fruit growth (with a mean error of 8% for fresh mass) and sugars (with a mean error of 19%) on plants

with different fruit loads under field conditions. The first estimates of a set of parameters which govern water fluxes and uptake are shown.

Finally, in Chapter VI we present a general discussion and conclusions. The chapter was organized to integrate the effect of SSR on: i) fruit responses; and ii) leaf responses. The chapter also presents the future directions concerning to the study of SSR in blueberry plants. The main conclusions of this thesis are:

i) The biomass allocated in fruits and leaves was affected by manipulation of SSR via pruning, which resulted in varying fruit load per plant. The changes in fruit quality traits, as measured of berry weight, dry mater and soluble sugar concentration, were in turn explained by changes in fruit load. An enhanced fruit quality is reached when fruit load was low, although yield is negatively affected. Source limitation to fruit size was mainly occurred at cell division development phase, as relative fruit growth rate decreased. The adapted ecophysiological model correctly simulates the seasonal differences in fruit growth and sugar concentration given by manipulating pruning. The modeling approach allows obtaining the first estimates of a set of biophysical parameters governing water fluxes and uptake.

ii) Source activity (leaf photosynthesis) is down-regulated by sink demand when leaves are subjected to low fruit load either at whole-plant scale or at fruiting shoot scale. The mechanisms underlying this effect were different according to the analyzed level. While a limiting CO_2 concentration in the stomata could be associated with the loss of CO_2 fixation capacity when SSR changed as effect of pruning, sugar-sensing mechanisms arise as driving force behind photosynthetic regulation to changing SSR at fruiting shoot scale.

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CHAPTER I
General Introduction

1.1 General introduction

The vegetative and reproductive growth of higher green plants well supplied with water and inorganic nutrients depends primarily on i) the accomplishment of photosynthesis and ii) the transport of organic compounds from source of assimilates to heterotrophic cells which constitute metabolic sinks (Beevers 1969). Historically, researchers on plant physiology and metabolism have viewed the link between assimilation, storage and growth in terms of sink–source relationships (SSR) – the complex interplay of availability of supply and demand of carbon. Formally, the "source" can be defined as a photosynthesizing tissue or organ with substantial Rubisco activity and export of carbon skeletons and typically comprise all types of green leaves, once they become carbon-autonomous or autotrophic. The "sink" could be defined as a heterotrophic tissue or organ which requires import of carbon compounds. Typical examples for "sinks" are flowers, petals, fruits, shoots or roots, which normally have different ability for carbon uptake: Fruit >> flower >> root >> shoot >> leaf (Blanke 2009). In the source-sink system there are relatively rapids interactions where the activities of carbon source and sink organs seem to be closely co-ordinated such that a balance is maintained between the source of supply and the sink demand (Foyer et al. 1995; Génard et al., 2007). For example, leaf photosynthesis rate (*A*) is modified by the establishment of new sinks during plant growth, which in turn, depends on specific developmental process whose onset may be controlled by environmental factors such as photoperiod, temperature and light quality. Thus, during periods of low carbohydrate demand by sinks, a down-regulation of *A* activity of source organ can occur (Franck et al., 2006a; Gucci et al., 1994; Iglesias et al., 2002; Léchaudel et al., 2005; Palmer 1992; Quereix et al., 2001). Conversely, when sink demand is high, the competition for carbohydrates by competing sinks can subsequently lead to a reduced vegetative growth, which results in a source-limitation to growth of sink organs and higher *A* to counterbalance lower whole-canopy leaf area. This is the case of several deciduous and evergreen tree crops, such as vines where leaf area, leaf size, shoot length, node number and internode length were inversely

related to fruit load (Edson et al., 1995); and coffee and olive plants where shoot elongation and inflorescence number is strongly reduced on heavy fruit-bearing trees often leading to alternate bearing pattern (Cannell 1971; Franck et al., 2006b, Haouari et al., 2013).

The assimilate supply is regulated by the sink itself (Paul and Foyer, 2001). The photosynthetic machinery represents a huge investment of resources and it is logical that the extent of this investment responds to the economy of the whole plant in order to maximize the use of available light, to minimize damaging effect of excess light and to optimize the use of limiting resources (Paul and Foyer, 2001). Carbon assimilation by photosynthetic tissues leads to carbon compounds accumulation in leaves which reflects the metabolic state of photosynthetic cell. These carbon compounds can serve to feedback/feedforward control the rate of photosynthetic carbon fixation via sugar-sensing mechanisms that lead to changes in gene expression including down-regulation of a large set of photosynthesis-related transcripts (Eberhard et al., 2008).

Because the source-sink balance is a complex system in which there are regulations due to feedback/feedforward mechanisms and interactions between different functions of the different plant compartments, simulation models have been developed during the last 30 years to summarize and quantify this complexity (Le Roux et al., 2001, Génard et al., 2007). These models have been powerful tools for analysing the impacts of source-sink balance from single leaf metabolism to whole plant performance. In fact, many studies dealing with plant quantitative traits are based on the analysis of whole models and/or their compartments and parameters (Granier et al., 2002; Tardieu 2003).

In Chile, fruit crops oriented to exportation market are cultivated under a high input condition, where diseases are fully controlled and irrigation and fertilizer supply is not a limiting factor. In these conditions, most fruit species tend to be very productive such that source-sink imbalances can occur, affecting the plants annual outcome with implications on fruit quality and biomass gain to support the following production cycle. Source-sink imbalances can be faced with

agronomic practices seeking to balance fruit and vegetative growth, either by manual or chemical fruit thinning or by increasing the vegetative growth through nitrogen supply, irrigation or pruning.

Unlike other fruit crops, research based on source-sink approach has been scarcely carried out in blueberry plants (*Vaccinium corymbosum* L.) despite that SSR of this crop is annually modified via dormant pruning, which regulates fruit load and plant architecture (Strik et al., 2003). Usually, pruning is slightly applied in blueberry orchards because growers seek the highest yield per surface area. This may induce in a source-limitation to carbon gain both to vegetative organs and individual fruits, conditioning long-term sustainability of this crop and fruit quality. This latter aspect becomes important due to both increasing demand for fruit quality, especially in Europe and USA under current regulations, and as a factor for increasing fruit price. In this context, growers will have to adapt their technical choices to increase fruit quality. Based on current knowledge, the SSR is the basis of any fruit production system and a good knowledge of this matter may allow improving blueberry cultivation and fruit quality. Such information in blueberry is scarce which has resulted in a limited understanding of the biological and physiological processes controlling yield and fruit quality of this crop.

This thesis proposes to study the effect of SSR on vegetative and productive responses, fruit quality and physiological and structural leaf traits of field-grown blueberry cultivar 'Brigitta'. Two work scales are used to study the effect of varying SSR: i) whole-plant scale through manipulating pruning intensity, and ii) fruit-bearing shoot, through fruit load adjustment and girdling. The corresponding and other available knowledge is taken to be integrated in a process-based model, which predicts growth and sugar accumulation of blueberry fruit.

1.2 Hypothesis

1. Pruning severity affects whole plant source-sink relationship in field-grown highbush blueberry (*Vaccinium corymbosum* L.) plants, inducing either sink limitation to leaf carbon assimilation capacity and yield when fruit-to-leaf area ratio decreases, or source limitation to fruit growth and quality when fruit-to-leaf area ratio increases.
2. Low sink demand limits leaf carbon assimilation capacity in field-grown highbush blueberry (*Vaccinium corymbosum* L.) plants by non-structural carbohydrates accumulation in leaves, which is accompanied to physiological and structural leaf traits rearrangements.

1.3 General goal

To evaluate the effect of varying source-sink relationship in a blueberry cultivar on its vegetative and productive responses, fruit quality and leaf traits under field conditions.

1.4 Specific goals

1. To study the effect of pruning severity on yield, leaf gas exchange variables and fruit load as driver of fruit quality traits.
2. To evaluate physiological and structural leaf traits of blueberry leaves subjected to different source-sink relationships at fruiting shoot scale.
3. To implement a process-based model to simulate growth and sugar concentration of blueberry fruits growing in plants subjected to different pruning severities.

CHAPTER II

Source-sink relationships in plants: A review with emphasis in fruit crops

2.1 Introduction

Plants must achieve a balance between carbon assimilation, carbon storage and growth, integrating and regulating its metabolic processes to maximize the use of available light, to minimize the damaging effects of excess light and to optimize the nutrients. Historically, researchers on plant physiology and metabolism have viewed the link between assimilation, storage and growth in terms of sink–source relationships (SSR), an interaction between carbon supply and demand by source and sink organs, respectively. Studies of source-sink relationships have been largely empirical in nature, but these have allowed predictions to be made of the consequences of change to either the supply or demand for photosynthate in many plants (Wardlaw, 1990). An organ is defined as a source or a sink according to the direction of net transport of assimilates associated with it. Thus, a source can be crudely defined as an organ that is a net exporter of carbon assimilates, whereas a sink can be defined as an organ that does not meet your own carbon requirements and therefore must export them from source organs. The assimilate fluxes from sources to sinks are dynamic on plant life cycle and characterized by source-sink transitions due to changes in the ability of sink organs for attracting photo-assimilated or in the number of sink organs competing for a common pool of sugars (Roitsch, 1999), which are in turn controlled by environmental influences such as photoperiod, temperature and light quality experienced by the plant.

In fruit crops, either source- or sink-limiting situations may exist. The competition for carbohydrates by competing fruits can subsequently lead to a reduced vegetative growth of shoots, which limits leaf area and in turn carbon supply for fruits growth and quality (Génard et al., 2006). On the other hand, sink limitation during periods of low carbohydrate demand by fruits due to either low fruit load or periods of low (or absent) fruit growth, causes down-regulation of photosynthetic activity of source organs (Lemoine et al., 2013). In either case, the plant annual outcome may be

affected: fruit production and quality and availability of C reserves and structure (branches, roots and fruiting sites) to sustain the following production cycle (George et al., 1995).

Because the source-sink relationship is a complex system in which there are regulations due to feedback/feedforward mechanisms and interactions between different functions of the different plant compartments, simulation models have been developed during the last 30 years to summarize and quantify this complexity (Le Roux et al., 2001; Génard et al., 2007) from single leaf metabolism to whole plant scale. In fact, many studies dealing with plant quantitative traits are based on the analysis of whole models and/or their compartments and parameters (Grossman and DeJong, 1994; Granier et al., 2002; Tardieu, 2003) even through most detailed three dimensional representation of canopy architecture used for up-scaling processes from the leaf scale to the whole plant scale, among them photosynthesis (Wohlfahrt et al., 1999).

This chapter aims to review aspects of carbon supply and demand by source and sink organs with special emphasis in fruit crops, as well as, the effects and mechanisms involved when either source- or sink-limitation occurs. Besides, how carbon supply and allocation has been conceptualized and integrated through modelling approach is also discussed.

2.2 Carbon supply

During plant growth and development, carbon is mainly supplied by source organs which show the ability of photosynthesizing. The assimilated carbon can be supplied by all tissues containing chlorophyll; however, leaves are the main source organ exporting up to 80% of photosynthetic fixed carbon at mature stage (Lemoine et al., 2013).

2.2.1 Leaves as main source organ

The leaf can export up to 80% of photosynthetic fixed carbon at its mature stage (Lemoine et al., 2013). Carbon assimilation is directly dependent upon the availability of radiation and varies greatly with changes in light intensity showing a curvilinear response to gradual increases in radiation (Fig. 1.1).

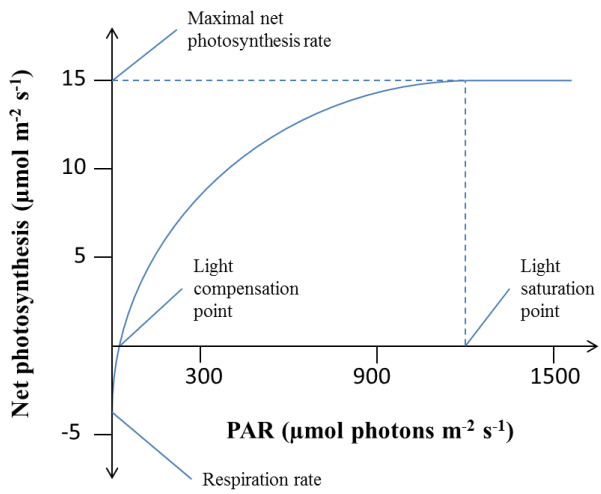

Figure 1.1 Typical photosynthetic light-response curves. Physiological parameters derived from the curve are shown.

At lower light regimes, the light curve reflects a net release of CO_2, because more CO_2 is given off by respiration than is fixed by photosynthesis. At somewhat greater light intensities the light compensation point is reached. At the light compensation point, photosynthesis fixes CO_2 equalling to the CO_2 released by respiration. Once the compensation point has been passed, CO_2 uptake increases rapidly. When light intensity increases to a very high level, photosynthesis continues to increase only slightly or not at all; and the rate of CO_2 uptake is now limited not by photochemical but rather by enzymatic processes, and by the supply of CO_2 (Larcher, 1980). The maximum rate of net photosynthesis or light-saturated photosynthesis (A^{sat}) by a plant at a given state of development and activity, under natural conditions of atmospheric CO_2 content and optimal

conditions with respect to all other external factors, is called photosynthetic capacity (Larcher, 1980).

The A^{sat} changes with species and even within the specie. Flore and Lakso (1989), summarized A^{sat} (in μmol CO_2 m^{-2} s^{-1}) for various fruit species such as the avocado (*Persea americana*, 4.8±2.4), orange (*Citrus sinensis*, 9.9±1.6), peach (*Prunus persica*, 13.3±3.8), pear (*Pyrus communis*, 20.2), grapevine (*Vitis vinifera*, 12.4±1.4), blueberry (*Vaccinium* sp., 12.7±7.4) and strawberry (*Fragaria x ananassa*, 13.9±2.9). Sun and shade leaves are a typical example of varying photosynthetic rates within the specie. Sun leaves differ from shade leaves primarily in their higher A^{sat} and the transition from the light-limited part to the light-saturated plateau is generally abrupt in shade leaves, but more gradual in sun leaves (Lambers et al., 2008). Although shade leaves typically have a low A^{sat}, they have lower light-compensation points and higher rates of photosynthesis at low light because their lower respiration rates per unit leaf area (Lambert et al., 2007). Contrasting to leaves acclimated to low irradiances, sun acclimated leaves have a high Rubisco-to-chlorophyll ratio, chlorophyll *a*-to-*b* ratio, xanthophyll carotenoids relative to chlorophylls and less stacking of the thylacoids (Terashima and Hikosaka, 1995; Lichtenthaler 2007). The rate of dark respiration typically covaries with A^{sat} (Lambers et al., 2008). The quantum use efficiency (as the initial slope of the light curve response) of both sun and shade acclimated leaves does not differ largely, except when shade-adapted plants become inhibited or damaged at high irradiance (photo-inhibition), which reduces it (Lambers et al., 2008).

Leaf age limits carbon supply from photosynthesis, with younger leaves having lower A^{sat} than old ones (Flore and Lakso 1989). The magnitude in which A^{sat} differs with leaf age depends on species. For example, the Figure 1.2 shows the seasonal variation of A^{sat} of apple leaves (Wünsche et al., 2005). The A^{sat} gradually increase (about 30%) from early season and reaches a maximum at leaf expansion. These higher rates gradually decrease (about 43% from the highest A^{sat} value) concomitantly to onset of senescence (Dickmann, 1971; Grosman and DeJong, 1994) that causes a

redistribution of resources, especially nitrogen, to younger leaves for optimization of whole-shoot photosynthetic income (Field and Mooney, 1983).

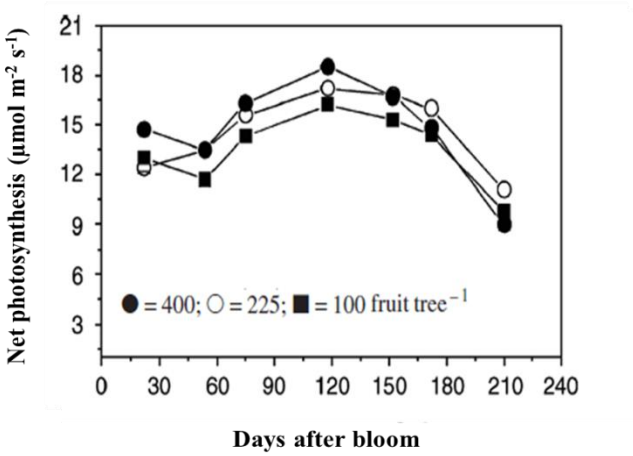

Figure 1.2 Seasonal net photosynthesis (µmol CO_2 m^{-2} s^{-1}) of 7-year-old apple trees cv. 'Braeburn' on rootstock M.26 apple trees for varying fruit load in New Zeland. Adapted from Wünsche et al. (2005). In the figure, net carbon exchange rate (at midday) steadily decreases from fruit harvest (about 120 days after bloom).

In contrast, no significant differences (n= 32, P=0.14, own unpublished results) were found between A^{sat} of younger (13.83 µmol CO_2 m^{-2} s^{-1}) and mature (14.5 µmol CO_2 m^{-2} s^{-1}) blueberry leaves cv. 'Legacy' growing in non-limiting conditions in the field (Fig. 1.3).

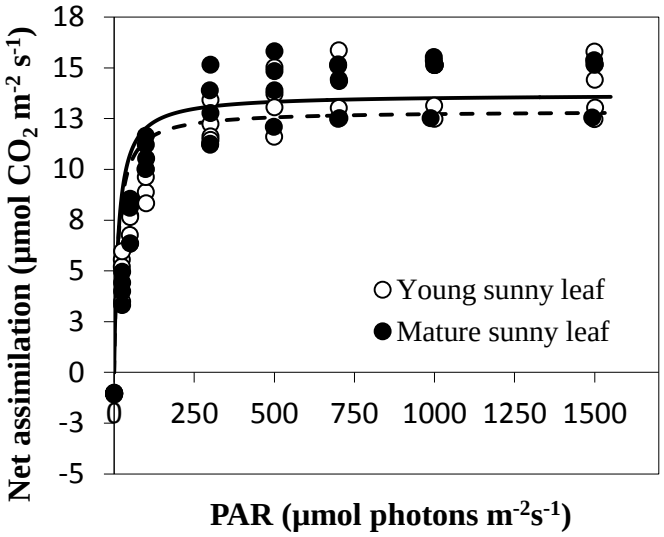

Figure 1.3 Net photosynthesis (µmol CO_2 m^{-2} s^{-1}) against photosynthetic active radiation (PAR) of younger and mature leaves of three year-old blueberry cultivar 'Legacy' under field conditions. Curves were constructed from morning to midday using portable photosynthesis system Li-6400 (LICOR, Nebraska, USA).

2.1.2 Carbon supply by organs other than leaves

Although leaves can export up to 80% of photosynthetic fixed carbon at mature stage (Lemoine et al., 2013), there is no doubt that photosynthesis of green tissues other than the leaf mesophyll such as fruits and stems positively contribute to the overall carbon budget of plants. In woody crops such as orange (Moreshet and Green 1980), avocado (Blanke 1992), blueberry (Birkhold et al., 1992), peach (Pavel and DeJong, 1993a) and coffee (Vaast et al., 2005), immature green fruits contribute notably to their own carbohydrate requirements for growth and maintenance. For instance, green berry coffee accounted for about 12% of the total daily berry carbon requirements at the bean-filling stage (Vaast et al., 2005), whereas blueberries fruit photosynthesis was estimated to contribute 15% of the total carbon required for fruit development (Birkhold et al., 1992). In the case of coffee, berry photosynthetic area accounted for about 20% of the total tree photosynthetic area in heavily cropped plants in early phase of bean-filling (Vaast et al., 2005).

Stem surfaces of woody plants may be equivalent to one half or more of the leaf surface (Mooney 1972). Stem photosynthesis has been studied in various tree species (Pfanz et al. 2002). Stem has a reduced efficiency of gas exchange than leaves, however, it would operate under somewhat more severe conditions than the leaves can tolerate (Mooney 1972), for example deciduous woody trees with chlorophyllous cells masked with a minimal corky or other protective tissue could be photosynthetically active in winter (Berveiller et al., 2007). The apparent gross photosynthetic rate in saturating light was 3.73 $\mu mol CO_2\ m^{-2}\ s^{-1}$ in *Alnus glutinosa* in summer (Berveiller et al., 2007).

Apart from those photosynthesizing tissues, store carbon reserves become an important source of carbon to support the initial growth of tissues; especially in deciduous plants and seeds. Carbohydrates stored in tissues adjacent to the translocation pathway are used for growth when remobilized. This is especially true for deciduous woody plants, which rely on stored carbon reserves in their woody tissues to support the expanding buds during this period of high carbon demand (Sprugel 2002). For instance, in 'Bonnita' southern blueberry, Darnell and Birkhold (1996) reported that root starch concentration dropped by 83% from dormancy to anthesis and stem and root sugars by 63 and 66 % for the same period, respectively. In vines cv. 'DeChaunac' McArtney and Ferree, (1999) informed that root starch concentration declined until 50% during the first 5 weeks after budbreak and then increased due to the shoots probably became photosynthetically autonomous (McArtney and Ferree, 1999).

2.1.3 Modelling carbon supply

Different approaches have been described to simulate carbon supply, which vary in their complexity. The simplest approaches for simulating photosynthesis are based on empirical functions, which relate the carbon assimilation response of a single leaf to increasing fluxes of photosynthetically active radiation (PAR) i.e. light-response curve, as previously defined (see Fig. 1.1). In these models, photosynthesis is affected by sink strength (Léchaudel et al., 2005) or by the

amount of reserves in leaves (Lescourret et al., 1998), which can down-regulates photosynthesis. Although parameters derivate from light response curve are important factors for understanding the physiological status of leaf, the empirical models of photosynthesis consider a limited number of parameters in order to simplify model framework (Table 2.1).

Table 2.1 Some empirical leaf photosynthesis approach used for modelling carbon supply.

Equation	Model type	Reference
(1) $A_n = \frac{(k \cdot PAR - A^{\max}) - \sqrt{(k \cdot PAR - A^{\max})} - 4 \cdot \theta \cdot k \cdot PAR}{2 \cdot \theta} - R_d$	non-rectangular hyperbolic	Prioul and Chartier, 1977
(2) $A_n = A^{\max} \cdot \left(1 - C_0 \cdot e^{\frac{-k \cdot PAR}{A^{\max}}}\right)$	Exponential	Bassman and Zwier, 1991
(3) $A_n = A^{\max} \cdot \left(1 - e^{-a \cdot (PAR - LCP)}\right)$	Exponential	Prado and DeMoraes, 1997
(4) $A_n = (A^{\max} + R_d) \cdot \left(1 - e^{\left[-kPAR/(A^{\max} - R_d)\right]}\right) - R_d$	Monomolecular	Goudriaan, 1982
(5) $A_n = \frac{A^{\max} PAR}{(\rho + PAR)} - R_d$	Rectangular hyperbolic	Givnish 1988
(6) $A_n = \frac{k \cdot PAR \cdot A^{\max}}{k \cdot PAR + A^{\max}} - R_d$	Rectangular hyperbolic	Thorney, 1998
(7) $A_n = A^{\max} \cdot \left(1 - e^{-k \cdot PAR)}\right) - R_d$	Monomolecular model	Causton and Dale, 1990

Equation parameters; A^{max}: light-saturated photosynthesis (µmol CO_2 m^{-2} s^{-1}); R_d: dark respiration; k: apparent quantum yield, θ: convexity of the equation; *LCP*: light compensation point; *a and β (*as correspond): empirical parameters; γ: calculated as the ratio of a and A^{max} (Eq. 3); ρ: light saturation constant (defined as one half of the saturating PAR); C_0: index that reflects net photosynthetic rate approaching zero at a very weak irradiance.

The photosynthesis model proposed by Farquhar et al. (1980) represents the most physiologically sound approach presently available (LeRoux 2000). The Farquhar's mechanistic model estimates the intrinsic photosynthetic capacity of C_3 leaves as a function of leaf irradiance, intercellular CO_2 concentration and leaf temperature and includes two biochemical factors that can limit carbon assimilation (A_n, µmol CO_2 m^{-2} s^{-1}):

$$A_n = \left(1 - \frac{\Gamma^*}{C_i}\right) \min\{W_c, W_j\} - R_d \qquad (1)$$

where, Γ* is the CO_2 compensation point; C_i is mesophyll CO_2 concentration, W_c and W_j are the rates of carboxylation limited by Rubisco activity and by Rubisco regeneration, respectively; and R_d is respiration. Equation parameters are defined such as:

$$W_c = \frac{Vc_{\max} C_i}{C_i + +K_c(1+O/K_0)} \tag{2}$$

where, Vc_{max} is the maximum carboxylation rate; K_c and K_0 Michaelis-Menten constants for carboxylation and oxygenation, respectively; and O is intercellular O_2 concentration.

$$W_j = \frac{J}{4+8\Gamma^*/C_i} \tag{3}$$

where J is the potential electron transport rate (µmol m^{-2} S^{-1}).

The effect of nitrogen on photosynthesis can be easily introduced in Farquhar's model because the three key parameters of the model (the maximum carboxylation rate, the light-saturated rate of electron transport, and the dark respiration rate) are proportional to the amount of leaf nitrogen on an area basis (Field and Mooney, 1986; LeRoux et al., 1999; LeRoux et al., 2001). Because C_i is a model input, an estimate of stomatal conductance is required. For instance, C_i is computed by an empirical function of PAR and air CO_2 concentration in the model SIMWAL (Le Dizès et al., 1997), whereas Webb et al., (1991) by semi-empirical functions which include environmental and physiologic factors such as vapour deficit pressure and water leaf potential.

2.1.3.1 Up-scaling from leaves to canopy

By definition, canopy photosynthesis (A_c) is equal to the integrated sum of photosynthesis by leaves throughout the canopy volume (Baldocchi and Amthor, 2001). Estimating photosynthesis at the whole canopy level requires estimating the amount of light intercepted by the canopy. This is why modelling light penetration through a canopy and light absorption by foliage is crucial for the determination of A_c. Radiation attenuation through canopies can be described by Beer's law (Monsi and Saeki 1953) which has been used in several models incorporating the source-sink approach (e.g.

DaSilva et al., 2011; Fleisher et al., 2010; Luan et al. 1996; Wermelinger et al., 1991). Many researchers have reported radiative transfer theory for plant stands with horizontally homogeneous canopies (Nilson, 1971; Ross, 1981; Thornley and Johnson, 1990), which have allowed estimating A_c representing the canopy as a 'big-leaf' (Thornley and Johnson, 1990). In closed canopy with dense foliage distribution and with one species, the assumption of one dimensional random foliage distribution does not produce excessive errors (Norman and Javis, 1975). In the case of discontinuous canopies such as fruit crops with wide spacing and most plants in their earlier growth stages, this approach can lead to an under- or over-estimation of canopy performance. For discontinuous canopies, some other efforts have considered the non-random distribution of foliage over the vertical or horizontal direction with simple equations. In this situation, a discontinuous canopy is assumed to be made up of a group of foliage of a given shape, where the canopy can be divided into *n* layers (multi-layer) with many different leaf angle classes allowing simulating the impact of spatial gradients of microclimatic variables on the system of equations defining leaf photosynthesis (Baldocchi and Amthor, 2001). For plants with wide spaces both within and between rows, each isolated plant may be treated as a geometrical object (e.g. Mariscal et al., 2000; de Pury and Farquar, 1997) or as a foliage group (Norman and Welles, 1983). Foliage groups, such as foliage ages and light exposure (i.e. sunlit or shaded leaves), have also been considered (Higgins et al., 1992; de pury and Farquar, 1997; Garcia de Cortazar et al., 2005). When fruiting branch was considered as functional sub-unit in the model proposed by Leacourret et al. (1998) for peach, sunlit or shaded leaves classes were considered.

2.3 Carbon demand by sinks

Sinks include root and shoot apical meristems, young expanding leaves, cambium and developing fruits. The phloem itself is often considered to be a sink, although only a minor one in relation to the other sinks of the plant (Clifford, 1992). In terms of assimilate transport, carbon demand by sinks or sink strength (Clifford, 1992) is the ability of a sink organ to import assimilates

(Ho, 1988), which is in turn the end result of the product of two components: sink capacity and sink activity (Warren Wilson, 1972). While sink capacity of an organ is a measure of its size in terms of dry matter (for example in g), sink activity is a measure of the relative growth rate of the sink (g g^{-1} t^{-1}). Thus, sink capacity can be considered as a physical constraint, while sink activity as a physiological constraint upon the ability of a sink to achieve its full or potential strength or demand i.e. sink activity is a measure of how well a sink can mobilize assimilates. This may involve the action of growth regulators such as auxin, cytokinin, and abscisic acid, and key enzymes such as sucrose synthase, starch phosphorylase (Paul and Foyer, 2001).

Because the competitive ability of a sink organ for attract assimilates from a source organ depends on the organ itself and its development stage as well as on the development stage of other growing organs within plant, a priority system for unloading of assimilated occurs among sinks. Evidence for a hierarchy of sinks has been obtained via experiments where leaves and/or number of growing organs are manipulated. As general conclusion, fruit and seed growth dominate the growth of vegetative tissues, although flowers in contrast to fruits appear to be poor competitors (Wardlaw, 1990 and references therein). Whole-canopy growth generally dominates that of the roots, but many underground storage organs have the same ability as fruits to dominate the supply of photosynthates (Wardlaw, 1990 and references therein). However, the timing of organ initiation and development are key factors that will regulate both the competitive ability of a sink organ and carbon partitioning within a plant. In tomato, the sink strength of the inflorescence increased from flowering to fruiting stage, and the priority between sinks for assimilate in the order of roots > young leaves > inflorescence in a flowering plant changed to the order of fruit > young leaves > flowers > roots in a fruiting tomato plant (Ho, 1988).

The spatial position of a sink also influence the competitive ability of it for assimilates. In tomato truss, the import rate of the early-set fruit in the proximal position is greater than that of later-set fruit in the distal position (Ho et al., 1983), whereas in annual crops such wheat, grains on

positions closest to the rachis (proximal grains) resulted in higher weights than distal grains (Calderini et al., 1999).

At organ scale, the demand for assimilates of individual fruits as measurement of growth rate, change in response to their development stage and is generally greatest during cell enlargement in fruit such as cherry (Flore and Layne, 1999), peach (Génard et al., 2007) and blueberries (unpublished results) (Fig. 1.4).

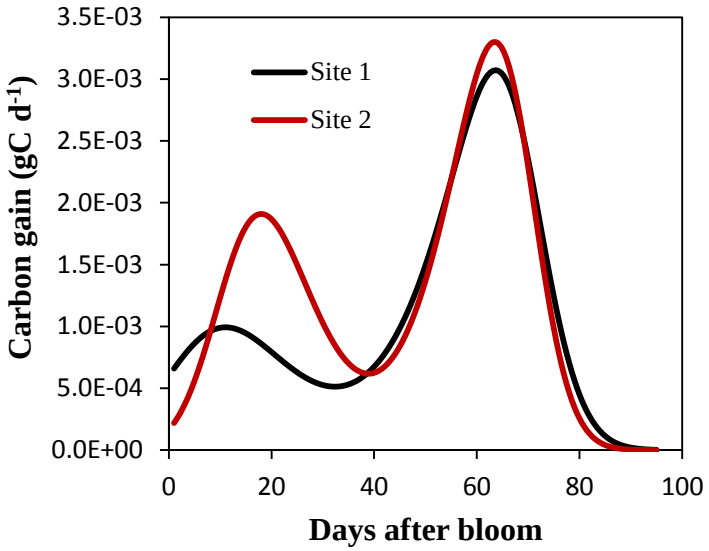

Figure 1.4 Carbon gain of blueberry fruit cv. 'Brigitta' from plants bearing low fruit load (see Chapter V). Data estimated by derivation of carbon accumulation curve. Inflection points indicate maximal gain at first and third fruit development stage. Site 1: Lautaro, Chile (38º29' S 72º 23' W) and, site 2: Freire, Chile (38º58'S 72º47' W).

The changes in sink demand at the cell, organ, and whole-plant levels are modulated by signaling molecules and/or global regulators of gene expression (Eveland and Jackson, 2011). These affect the biochemical control of carbon metabolism which, in turn, regulates carbon partitioning in sink regions (Geiger and Servaites, 1994). For example, sugars act like hormones and translating nutrient status to regulation of growth and the floral transition (Eveland and Jackson, 2011 and references therein) and modulate developmental genes implied in induction of potato tuber (Simko, 1994). The interactions between carbohydrate levels and plant growth regulators (especially auxin/ sugar antagonisms) and other essential nutrients such as N or P are also consistent

with this suggestion (Koch 1996). Regulation of the time and site of the transcription of specific genes controls the locations and levels of enzymes that, in turn, regulate the partitioning of carbon to growth, metabolism and accumulation of carbon at specific times and sites (Geiger et al., 1996).

On the other hand, changes in sink strength occur accompanied to changes in unloading pathways feeding it. Although this has been not amply studied, in sinks like developing seeds, symplastic discontinuity requires an apoplastic step for the transfer of photo-assimilates (Lamoine et al., 2013). A switch from apoplastic to symplastic unloading was noted during potato tuberization (Viola et al., 2001). In fruit development, contrasting results have been found: in grape berry, Zhang et al. (2006), demonstrated a shift from symplastic to apoplastic unloading whereas in apple and cherry fruit there is evidence for an apoplastic step in sucrose and sorbitol unloading, involving transporters (Gao et al., 2003; Zhang et al., 2004).

2.3.1 Modelling carbon allocation

The carbon allocated among sinks is also a major factor in plant productivity. Based on the harvest index i.e. ratio of harvested dry weight over plant dry weight (or above-ground shoot dry weight) a high harvest index indicates that a large amount of photo-assimilates has been diverted to the sinks harvested (Gifford et al., 1984). Biomass allocation within plants is affected by environmental surrounding experienced by the plant. In plant models, the factors usually taken into account, through their impact on growth dynamics are incoming radiation, soil nitrogen and water availability (LeRoux, 2001).

Carbon allocation modeling based on SSR, assimilate fluxes and allocation are assumed to depend on the respective ability of the different sinks to import available assimilates from the sources. In models, this ability, or “sink strength” (Farrar, 1993), is generally defined as the net flux (g C unit time^{-1}) that is imported into a sink under particular, often “non-limiting” conditions; specific rules allow then to compute the actual fluxes imported under the current conditions

(Lacointe, 2000). Theoretically the sink ability to import and use available assimilates should encompass both carbon deposition as new dry matter and carbon losses as respiratory CO_2 (Lacointe, 2000). Within these types of models we can find: i) hierarchical and ii) proportional models (Le Roux, 2001, Lacointe, 2000). In the hierarchical approach one can establish a priority ranking among different sink organs where the higher hierarchical level is the first to receive assimilates and successively so forth until reaching the organs with less priority. For example the 'PEACH' model (Grossman and De Jong, 1994) assumes, that having satisfied the necessities of maintenance respiration, the fruits, leaves, buds and branches have the same priority, followed by the trunk and finally the roots. Lakso et al., (2001) in apple trees, the highest priority level is given to the buds, then fruits and finally, on a same level, the roots and reserve structures. The 'VIMO' model (Wermlinger et al. 1991) considers that the level of partition changes during the growing season. Before the blooming season, the vegetative and reproductive growth (inflorescences) has the same priority; however that changes when the priority is taken by the reserve organs.

Proportional models distribute assimilates according to its demand (Génard et al., 2007, Marcelis and Heuvelink, 2007). Here, there is an intervention from the gross amount of exported carbohydrates from a source and the affinity of the sink where a decreasing function is used among the participating organs, following a metric topologic pattern; a defined distance between two points (for example, the bud-leaf distance) (Génard et al., 2007). The model proposed by Balandier et al., (2000) for walnut trees (SIMWAL) uses this approach.

Finally, based on SSR approach, modelers have connected the carbon fluxes to fruit quality traits. Nevertheless, fruits cannot be restricted to their carbon content or dry mass just because the water is their main component (Génard and Lescourret, 2004). Only a few models have been proposed to simulate water accumulation in the fruit. Fruit growth has been calculated by integrating numerically the equation for water balance, using water uptake and transpiration per unit of fruit area as a constant (Lee, 1990) or a variable (Génard and Huguet, 1996). Fishman and Génard, (1998) had proposed a model of fruit growth integrating both the dry matter and the water

accumulation within the fruit, which opened the way to considering the edible quality. In the model, the parent plant supplies the fruit with water and sugars, which are brought through xylem vessels and phloem sieve tubes. The magnitude of carbon and water supply is depending on SSR in the fruiting branch. The fruit consumes carbon and water through the respiration and transpiration processes. Finally, fruit fresh and dry weight and fraction of sugars accumulated in fruit has been correctly simulated in peach fruit (Génard et al., 2009). Because the model has a generic framework, it has been tested with modifications in several other fruits (Bar-Tal et al., 1999; Lechaudel et al., 2007; Liu et al., 2007; Quilot et al., 2005).

2.4 Regulations between source and sinks

In plants, the activities of source and sink organs appear to be closely co-ordinated such that a balance is maintained between carbon supply and demand (Foyer et al. 1995; Wardlaw 1990). This balance may be unfavourable when carbon assimilation rate is lower than the capacity of dry matter accumulation in other parts of the plant (source limitation) or when carbon assimilation is down-regulated by lower sink demand (sink limitation). In crop production, either source- or sink-limiting situations may exist (Ho, 1988), which may affect the plant annual outcome: total biomass accumulation, total fruit production and availability of C reserves and structure (leaves, roots and fruiting sites) to sustain the following production cycle.

2.4.1 Source-limitation to carbon gain

When C demand by competing sinks is high, a source limitation to carbon gain thereby reducing vegetative growth i.e. an increase in number of reproductive sinks increases the reproductive-to-vegetative ratio (Marcelis 1996). A high reproductive-to-vegetative ratio implies a reduced leaf area and light interception for photosynthesis thereby exacerbating source limitation to carbon gain. This is typically the case of coffee, where branch growth and development is strongly

reduced on heavy fruit-bearing trees often leading to branch dieback and resulting in a strong alternate bearing pattern (Cannell 1971; Franck et al. 2006b). The effect of limiting source carbon supply has also been documented in species like vines where lower grape growth and yield (Candolfi-Vasconcelos and Koblet, 1990; Foyer et al., 1995) and lower leaf area, leaf size, shoot length, node number and internode length (Edson et al., 1995), resulted from defoliation and higher fruit load, respectively. In order to counterbalance lower whole-canopy leaf area and thus the loss of photosynthetic potential, an enhanced carbon assimilation capacity by leaf (A_n) is normally observed. In 'Sharpblue'southern blueberry (*Vaccinium corymbosum* L.) plants, while whole-canopy assimilation rate and leaf area decreased as fruit load increased in fruiting branches, A_n increased from 3.8 to 10.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Maust et al., 1999a). In similar way, while whole-canopy assimilation rate decreased 10% in heavily cropped apple trees, A_n increased 44% (Wünsche et al., 2000).

One of the main effects observed on source-limited fruit trees is the lower fruit quality obtained (e.g. Intrigliolo and Castel, 2010; Reginato et al., 2007; Vaast et al., 2006). For example, our own unpublished results showed that decreasing source-limitation by growing leaf-to-fruit ratios obtained via management of pruning intensity, resulted in enhanced fruit quality as fruit dry matter and sugar concentration increased (Fig. 1.5).

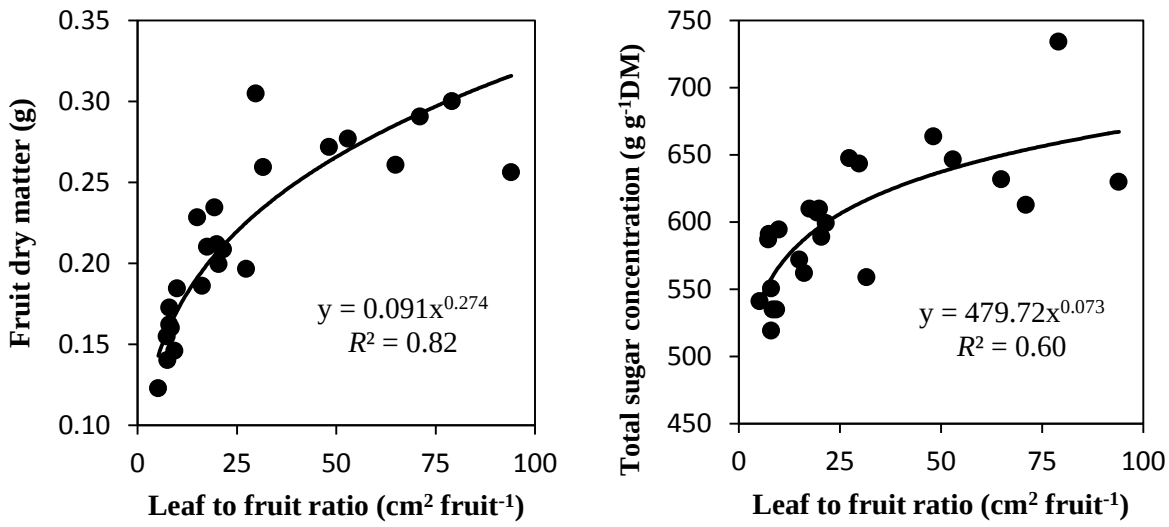

Figure 1.5 Berry weight and sugar concentration of 'Brigitta' blueberry fruits plotted against leaf-to-fruit ratio. Data were obtained from 4-and 5-year-old plants subjected to different pruning severity in field conditions. DM= dry matter.

Fruit weight= $\sum_{k=m}^{n}\left(\frac{Weight\ of\ subsample}{\#of\ fruit\ of\ subsample}\ x\ \frac{Weight\ harvest\ date}{Total\ yield\ of\ the\ season}\right)$, sum starts at m (first harvest) and ends at *n* (last harvest). Total leaf area (*LA*) was estimated from subsample leaf area such as:

$LA\ (cm^2) = \frac{Total\ leaves\ dry\ weight\ x\ LA\ subsample}{Leaves\ dry\ weight\ subsample}$. Total leaves dry weight was obtained from completely three defoliated plants per treatment after fruit harvest. Number of fruit was estimated by division of yield and mean fruit weight. Soluble sugars were obtained from samples using high performance thin layer chromatography (for more details see Chapter III).

A source limitation can induce to up-regulation of genes controlling photosynthesis and carbohydrate remobilization and export, while decreasing mRNAs for carbohydrate storage and utilization (Koch, 1996). Such a kind of mechanism has been reported for citrus in which carbohydrate starvation due to alternate bearing resulted in up and down-regulation of genes involved in carbon assimilation, translocation and storage (Kim and Lieth, 2003). These regulations in citrus could also be achieved by changing the source-sink balance of plants either by removing fruit through thinning or reducing carbon supply through shading (Kim and Lieth, 2003).

2.4.2 Down-regulation of leaf carbon assimilation by sink-limitation

The effect of sink on source organs has been analysed with special focus in A^{sat}. The 'end-product inhibition of photosynthesis' hypothesis has been tested from many time ago (Boussingault, 1868). However, the literature shows that the negative correlation between photosynthesis and end-product inhibition of photosynthesis is still not readily observable with all plants tested (e.g Goldshdmidt and Huber, 1992; Nebauer et al., 2011). For example, low sink demand after cucumber fruit removal decreased photosynthesis rate without influencing accumulation of sugars in leaves (Marcelis, 1991). A similar situation is observed latter in grapevines leaves under a low sink demand (Chaumont et al., 1997). This last authors have hypothesized that down-regulation of photosynthesis could be related to an increased phloematic carbohydrate content (Quereix et al., 2001). Conversely, Roper (1988) found that low sink demand induced sugar accumulation in sweet cherry leaves without decrease photosynthesis. Despite above-mentioned reports, the Boussingault's hypothesis has been confirmed in several fruit crops, even between genotypes. It is interesting to notice that the high genotypic differences observed in *A* of peach leaves by Quilot et al. (2004), is not related to the variation of the potential photosynthesis, which is very similar between genotypes, but to differences in fruit sink strength. Indeed genotypes with low fruit sink strength accumulate reserves in the leaves, which depress the actual A_n through a feedback mechanism (Quilot et al., 2004).

Three mechanisms have been mainly explored to explain down-regulated photosynthesis by end-photosynthesis products: i) inhibition by starch; ii) gene regulation by sugar signals and ii) phosphate availability.

i) *Inhibition by starch*. In most plants, a large fraction of photo-assimilated carbon is stored in the chloroplasts during the day as starch and remobilized during the subsequent night to support metabolism. When sink demand is low, a high starch levels in the source could be accumulated leading to a severe impairment to chloroplast structure and function which may result in reduced

photosynthetic rates (Paul and Foyer, 2001; De Groot et al., 2003). Bondada and Syvertsen (2005), demonstrated via microscopy that most of the membrane system in the form of grana and stroma lamellae was pressed against the chloroplast envelope by the starch granules in nitrogen-limited citrus leaves. This was accompanied with a degradation of the membranes. Similar results were observed by Etxeberria et al., (2009) who induced changes in source-sink relation by mean of a bacterial disease that accumulated high levels of starch. Considering that a mechanism controlling CO_2 diffusion from the intercellular air spaces to the chloroplast stroma seems to be related to the chloroplast movement (Makino and Mae, 1999), if chloroplast is pressed by starch its movement can be disrupted, leading either to a decreasing surface area adjacent to the plasmamembrane and a decreasing CO_2 conductance (von Caemmerer and Evans, 1991) and damage and disorientation of grana and thylakoids (Bondada and Oosterhuis, 2003), which reduces A_n, concomitantly. Although little evidence supports this hypothesis, recent works in Arabidopsis mutants reveals that the accumulation of maltose and malto-oligosaccharides causes chloroplast dysfunction, which may by signalled via a form of retrograde signalling and trigger chloroplast degradation (Stettler et al., 2009).

ii) *Phosphate availability*. When sink demand is low, photosynthesis could be restricted by a lack of free orthophosphate in the chloroplast (Du et al. 2000). Under conditions of low demand by the sink, sucrose synthesis is usually reduced, and less phosphate is in turn available for exchange with triose phosphate from the chloroplast (via the phosphate translocator). If starch synthesis, which releases orthophosphate in the chloroplast, could not release phosphate fast enough, a deficiency in phosphate would ensue, ATP synthesis and CO_2 fixation would decline. A study with potato plants transformed with antisense DNA to the phosphate translocator provides support for this hypothesis (Riesmeier et al., 1993). The transformed plants, which displayed reduced phosphate translocator activity, allocated proportionately more carbon into starch and less into sucrose. These effects were accompanied by a reduction in the light- and CO_2-saturated rates of photosynthesis in young plants.

iii) *Sugar signals regulating photosynthesis genes*. High sugar levels decrease the transcription rate and expression of genes for many photosynthetic enzymes (Koch 1996). The changes in gene expression occur over the same time frame as the source adjustments already described. For example, in source leaves of spinach (*Spinacia oleracea* L.), mRNA for several photosynthetic enzymes decreased when soluble carbohydrates accumulated as a result of inhibition of export from the leaf (Krapp and Stitt, 1995). Although transcript levels began to decline almost immediately, changes in photosynthetic enzyme activity were apparent only after several days. In this species at least, photosynthesis appeared to be inhibited because of changes in gene expression, not because of phosphate limitation, as discussed earlier.

On the other hand, the effect of carbohydrate accumulation on photosynthesis differs along leaf development. Krapp et al. (1991), fed a 50 mM glucose solution to sink and source spinach leaves from their petioles via the transpiration stream. The photosynthetic rate per leaf area of the glucose-fed source leaves was 30% of that of the control source leaves, while that of the glucose-fed sink leaves was not different from that of the control sink leaves. In the same way Araya et al. (2006), reported that the A^{sat} of young sink leaves was similar between the sugar-treated and control leaves (no sugar-treated) at an ambient CO_2 concentration, whereas A^{sat} of sugar-treated source leaves was significantly reduced than control bean source leaves. Finally, leaf habit would also have impact on susceptibility to down-regulated photosynthesis by low sink demand. Franck et al. (2006b), hypothesized that in the case of coffee, the sharp increase of carbohydrate content in leaves accompanied with a important down-regulation of photosynthesis is results of high investment of carbon in leaf area at the expense of investment in woody tissue (i.e. tissue with reserve accumulation capacity). Because of this restricted capacity for carbohydrate storage in woody tissues, these authors suggested that leaves would play a more important role in carbon storage in such species than in species which invest less carbon in leaves, as deciduous fruit trees. This would make evergreen species more prone to sink limitation to photosynthesis (Franck et al. 2006b). A similar hypothesis has been reported concerning nitrogen balance by Warren (2004), who

suggested that evergreen leaves have an important role in nitrogen storage which may explain their poorer photosynthetic performance than deciduous leaves (Ellsworth and Reich, 1992).

2.5 Conclusion and final remarks

In this Chapter, we reviewed concepts of carbon supply and demand, as well as, the effects and mechanisms involved when either source- or sink-limitation occurs. Besides, how carbon supply and allocation has been conceptualized and integrated through modelling-based approach was also discussed. Source organs, mainly leaves, produce assimilates, which are translocated to non-photosynthetic organs, known as sinks. In the source-sink system there are relatively rapids interactions where the activities of carbon source and sink organs seem to be closely co-ordinated such that a balance is maintained between the source of supply and the sink demand. In fruit trees, either source- or sink-limiting situations may exist, which may affect the annual outcome of plants: fruit production and quality and availability of C reserves and structure (branches, roots and fruiting sites) to sustain the following production cycle.

Based on current knowledge, the SSR is the basis of any fruit production system and a good knowledge of this matter may allow to improve fruit cultivation as well as to increase understanding of the biological and physiological processes controlling yield and fruit quality. This becomes significant due to the present concerns about sustainable horticulture and fruit quality. Although the empirical knowledge based in SSR has been important to improve fruit cultivation and quality, it takes time to be established in the field. For this reason, advances in SSR based-models should be led to establish support decision systems, which have potential for evaluating agro-technical management in the short time. On the other hand, the use of models also allows predicting the impact of climatic conditions on crops, which is an important factor to consider, taking account the current predictions regarding climate change.

CHAPTER III

Pruning severity affects yield, fruit load and fruit and leaf traits of 'Brigitta' blueberry (Vaccinium corymbosum L.*)*

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3.1 Introduction

In southern Chile, blueberry orchards are oriented to the export market and are regularly cultivated under high input conditions. In these production systems, most blueberry cultivars tend to be very productive as a result of a high proportion of photo-assimilates allocated to fruit bud formation in the previous season. Although a high number of fruit buds per plant is desirable to achieve higher yield per unit area, the competition for assimilates among fruits can reduce whole-canopy leaf area, which leads to a lower fruit quality (Léchaudel et al., 2005). In fact, fruit weight and soluble sugar concentration typically decrease when the ratio between fruit number and leaf area (fruit load) is high. This is because the carbon source offer (mainly leaves) fails to meet the demand of carbon sinks (mainly fruits) (Seehuber et al., 2011; El-Boray et al., 2013). In order to balance fruit load, pruning is a crucial practice in blueberry production, with a positive effect on vegetative growth and fruit weight (Strick et al., 2003). However, if pruning is severely applied, a low fruit-to-leaf area ratio will develop, which can have a negative impact on gas exchange variables as occurs in several fruit species (Lechaudel et al., 2005; Franck et al., 2006; Quentin et al., 2013).

Although the effect of pruning on blueberry fruit weight has been already reported (Strick et al., 2003), an approach based on fruit load has been not widely used to explain differences in fruit quality under field conditions. On the other hand, reports involving the physiological effects of pruning on leaf gas exchange variables are scarce in blueberry (Maust et al, 1999).

The aims of this study were 1) to examine the effect of different pruning severities on yield, whole-canopy leaf area and leaf gas exchange, and 2) to evaluate fruit load as a predictor of fresh weight, % dry matter and sugar concentration of blueberry fruit. Because periods in which increasing fruit load bring about reductions in the relative fruit growth rate (RGR) have been postulated as an indicator of a source limitation of fruit growth (Pavel and DeJong, 1993b), the

RGR was also estimated along the season. In order to reach the aims of this study, measurements were performed on field-grown highbush blueberry cv. 'Brigitta' (*Vaccinium corymbosum* L).

3.2 Material and methods

3.2.1 Plant material and treatments

The experiments were carried out on a commercial blueberry orchard located in the Araucania Region, Chile (38º29'S 72º 23' W). Plants were established at a spacing of 3 × 0.9 m, in north–south oriented rows on an Andisol. Fertilizer was applied to achieve 70 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1} and 75 kg K_2O ha^{-1}. Irrigation was no limited in the orchard and control of pests and diseases were applied according to the locally recommended practices. In winter 2009 and 2010, three pruning severities were applied on 48 four- and five-year old blueberry plants cv. 'Brigitta' arranged in a randomized complete block design consisting of four replicated blocks distributed in two orchard rows. Treatments corresponded to: slight, conventional and severe pruning, which were applied according the criteria described by Strik et al. (2003). All plants were first slightly pruned and then fruit buds were counted. Thereafter, conventional and severe pruning were applied and fruit buds per plant were adjusted targeting that conventionally and severely pruned plants had close to 50% and 20% of the fruit bud number present in slightly pruned plants (Table 3.1).

Table 3.1 Pruning treatments applied to 4-and 5- year old 'Brigitta' blueberry plants and fruit bud per plant resulting from pruning method. Percentage of fruit buds relative to slightly pruned plants is shown.

Treatment	Plant age	Fruit buds · $plant^{-1}$	Fruit buds relative to slightly pruned plants (%)
Slight	4-year old	664.66±49.72	100
	5-yerd old	799.69±11.98	100
Conventional	4-year old	353.83±35.51	53.23
	5-yerd old	416.83±38.51	52.12
Severe	4-year old	146.69±14.43	22.07
	5-yerd old	173.71±19.42	21.72

3.2.2 Fruit measurements

Fruits from each replicate were weighed every 5–8 days to estimate yield per plant. Mean berry weight was determined taking randomly 20-fruit sample per plant at each harvest date. Fruit number was estimated by division of yield and mean fruit weight. Samples were then dried at 65° C to a constant dry weight to determine % of dry matter in fruits.

Fruit soluble sugar concentration was determined on three 20 fruit-samples randomly collected in each treatment replicate when fruits had reached maturity, 100 days after bloom (DAB). Samples were frozen to -80ºC and then lyophilized. Sugars from ground freeze-dried fruits (50 mg) were extracted in 80% ethanol, containing maltose (3g L^{-1}) as internal standard, for 1h at 60 ºC, and in distilled water under the same conditions. Each extraction was followed by centrifugation at 13,000x g. The pooled supernatants were used for sugar analysis. Soluble sugar extracts were analysed by high performance thin layer chromatography (CAMAG, Muttenz, Switzerland).

Fruit growth was assessed by measuring equatorial diameter with a digital calliper (accuracy ±0.01 mm). Measurements were periodically made on 4-year old plants from corolla fall until the moment when berries reached full blue colour development. Six representative sun exposed clusters of fruits were selected from each treatment. In order to decrease the degree of development asynchrony among individuals only the five largest fruits within each cluster were used for growth determination, independently of their position within the inflorescence (modified from Godoy et al., 2008). For these data, an experimental non-linear regression (R^2= 0.97, n= 99) between fruit diameter (*di*) and fruit dry matter (DM_{Fr}) was applied in order to estimate seasonal increase of fruit dry matter:

$$DM_{\mathrm{Fr}} = 1.765x10^{-5} \cdot di^{3.397} \quad (1)$$

The equation was constructed from fruits randomly taken from neighboring plants other than those subjected to evaluation. Fruit equatorial diameter was measured and then individually dried at

65° C to a constant dry weight. From estimated fruit dry matter, the mean relative growth rate (RGR) was calculated as described by Millaleo et al. (2013):

$$\mathrm{RGR} = (\ln DW_{\mathrm{Fr}}2 - \ln DW_{\mathrm{Fr}}1)/(d_2 - d_1) \quad (2)$$

where $DW_{\mathrm{Fr}}2$ and $DW_{\mathrm{Fr}}1$ are the average fruit dry matter at subsequent dates d_2 and d_1, respectively.

3.2.3 Whole-canopy leaf area and leaf gas exchange measurements

Four representative plants per treatment were completely defoliated at the end of each harvest season to estimate the whole-canopy leaf area per plant (m^2). From defoliated plants, 100 g fresh leaves samples were taken and then scanned to estimate their leaf area using a program developed in our laboratory, which was previously validated by O'Neal et al. (2002). After scanning, samples were dried at 65° C to a constant dry weight along with the rest of collected leaves. The leaf area of samples was related with its dry matter in order to estimate whole-canopy leaf area from total leaf dry mass.

Photosynthetic light-response curves were obtained on 4-year old plants at 67 and 94 DAB. In each measurement day and treatment, six expanded leaves per treatment experiencing fully sun exposure were measured between 8:30 and 14:00 hr. Leaves were selected from fruit-bearing shoots similar in vigour, length and number of fruit per leaf (1-1.4 fruits per leaf) to test the hypothesis that leaves on these branches are not autonomous regarding carbon gain and their light-saturated assimilation rates are driven by whole-plant fruit load resulting from pruning. An infrared gas analyzer (Li-6400, LICOR, Nebraska, USA) connected to a broadleaf chamber and with automatic control of leaf temperature, photosynthetic photon flux density and CO_2 concentration, was used for measurements. Leaf temperature was set at 20°C and ambient CO_2 and H_2O vapour concentrations were used during measurements. For treatments, light-response curves were constructed by plotting net photosynthesis (A_n) against incident photosynthetic photon flux density (PPFD), which ranged from 0 to 1,500 µmol (photon) m^{-2} s^{-1}. Stomatal conductance to water vapour (g_s, mol H_2O m^{-2} s^{-1}) and intercellular CO_2 concentration (C_i, µmol CO_2 μmol^{-1} photon) were also recorded for each A_n

value. Causton and Dale's (1990) model was fitted to light-response curves in order to estimate light-saturated photosynthetic rate (A_e^{sat}, µmol CO_2 m^{-2} s^{-1}), dark respiration rate (R_d, µmol CO_2 m^{-2} s^{-1}) and maximal quantum use efficiency for CO_2 assimilation as the initial slope of the photosynthetic light-response curve (a_{light}, µmol CO_2 μmol^{-1} photon):

$$A_n = A_e{}^{sat}\left[1 - e^{(-a_{\mathrm{light}}\mathrm{PPFD})}\right] - R_{\mathrm{d}} \quad (3)$$

where A_n is net photosynthesis (µmol CO_2 m^{-2} s^{-1}) and PPFD is photosynthetic photon flux density [mol (photon) m^{-2} s^{-1}`].

3.2.4 Statistical analysis

One and two-way ANOVAs were performed to identify the effect of treatment and the interactive effect between treatment and plant age on studied variables. Fruit weight, dry matter and soluble sugar concentration were analyzed through regressions with fruit load as explanatory variable. To evaluate association among measured gas exchange variables, regression analysis was also performed. A Tukey test (P< 0.05) was used to separate means. All data analyses and methods used for fitting models were carried out with R software through R Commander (Rcmdr version 1.8-3 in R version 2.14.2), and 'nls' function (R version 2.15.0), respectively.

3.3 Results

3.3.1 Yield, berries per plant, fruit weight, leaf area and resulting fruit load

Yield and fruit number significantly increased with decreasing pruning severity (Table 3.2). For 4-year old plants, the slightly pruned plants had 1.3 times and 2.2 times higher yields than conventionally and severely pruned plants, respectively. For 5-year old plants, these differences slightly increased reaching 1.7 times and 2.6 times, respectively.

Table 3.2 Effect of pruning severity on yield, berry weight, fruit number, leaf area and resulting fruit load of 4- and 5-year old blueberry plants cv. 'Brigitta' in the field. Mean values of the treatments and standard errors are shown.

Treatment (T)	Plant age (PA)	Yield (kg plant^{-1})	Fruit weight (g)	Berries plant^{-1}	Leaf area (cm^{-2})	Fruit load (fruit cm^{-2} leaf area)
Slight	4-year old	3.72±0.14 bd	1.22±0.04 c	3077.7±204.4 c	2.62±0.04 c	1.19±0.26 b
	5-yerd old	4.72±0.18 d	1.15±0.09 c	4145.8±226.2 c	3.04±0.19 bc	1.41±0.27 b
Conventional	4-year old	2.82±0.32 bc	1.56±0.04 b	1801.2±191.2 b	3.45±0.06 bc	0.53±0.31 a
	5-yerd old	3.03±0.16 bc	1.53±0.07 b	1984.5±107.5 b	4.03±0.04 ab	0.50±0.35 a
Severe	4-year old	1.68±0.18 a	1.98±0.05 a	858.5±110.4 a	4.13±0.04 ab	0.21±0.24 a
	5-yerd old	1.94±0.39 ac	1.99±0.07 a	967.0±175.9 a	4.95±0.05 a	0.21±0.35 a
Effect of T		<0.001	<0.001	<0.001	<0.001	<0.001
Effect of PA		0.31	0.57	0.38	0.118	0.77
T X PA		0.23	0.85	0.025	0.79	0.31

The differences in berry number per plant were higher than those in yield due to the compensatory effect of pruning severity on fruit weight (Table 3.2). Thus, for 4-year old and 5-year old plants, berries per plant increased 3.5 times and 4.3 times, from severely to slightly pruned plants, respectively. A significant interaction between pruning severity and plant age was observed for berries per plant (Table 3.2).

Mean berry weight significantly varied with pruning method with slightly pruned plants having 40% smaller berries than severely pruned plants. No significant effect of plant age and pruning treatment x plant age interaction on berry weight was observed (Table 3.2). Total yield was negatively correlated with berry weight (R^2= 0.66, P< 0.001). Pruning severity significantly affected canopy leaf area, while plant age and the interaction between pruning treatments and plant age did not (Table 3.2). Thus, canopy leaf area increased by close to 60% from slightly to severely pruned plants, considering the mean value of both plant ages (Table 3.2). Treatments were effective in generating significant differences in fruit load with values ranging from 0.21 to 1.41 fruits cm^{-2} leaf area. Plant age and pruning treatment x plant age interaction did not alter fruit load.

3.3.2 Fruit load as a predictor of fruit quality

Berry weight and dry matter (%DM) were significantly and negatively correlated to fruit load, with fruit load accounting for over 70% of the variance for both variables (Figure 3.1A and B). As observed in figure 1A, the %DM decreased in lower extent than berry weight as fruit load increased. Thus berry weight decreased by 50%, whereas %DM by only 6% when comparing severely and slightly pruned plants.

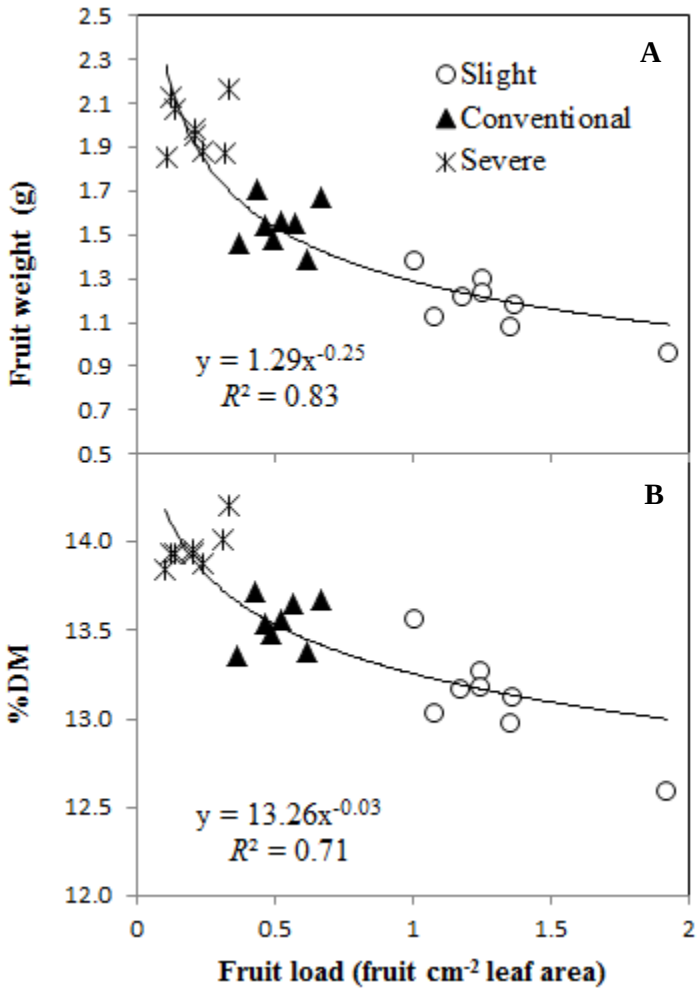

Figure 3.1 Fruit fresh weight (A) and fruit dry matter (%) (B) as a function of fruit load for 'Brigitta' highbush blueberry plants subjected to slight, conventional and severe pruning. Mean values of replicates (*n*= 4), non-linear regression and R^2 value are shown.

Sugars other than glucose and fructose were not detected in this study. Decreasing fruit load led to increased fructose, glucose and, thus, total sugar concentration in fruits (Fig. 3.2A, B and C). Soluble sugars were significantly related to fruit load through a potential fit with fruit load accounting for over 53% of the variance in sugar concentration. Total fruit sugar concentration increased close to 9% from slightly to severely pruned plants. Differences in fructose and glucose were similar to those observed for total sugar concentration. Glucose contributed more than fructose to the total soluble sugars; but differences between both sugars were not greater than 6%. The

%DM in fruits was positively and significantly correlated to the increment in total soluble sugars (R^2= 0.43, P< 0.01).

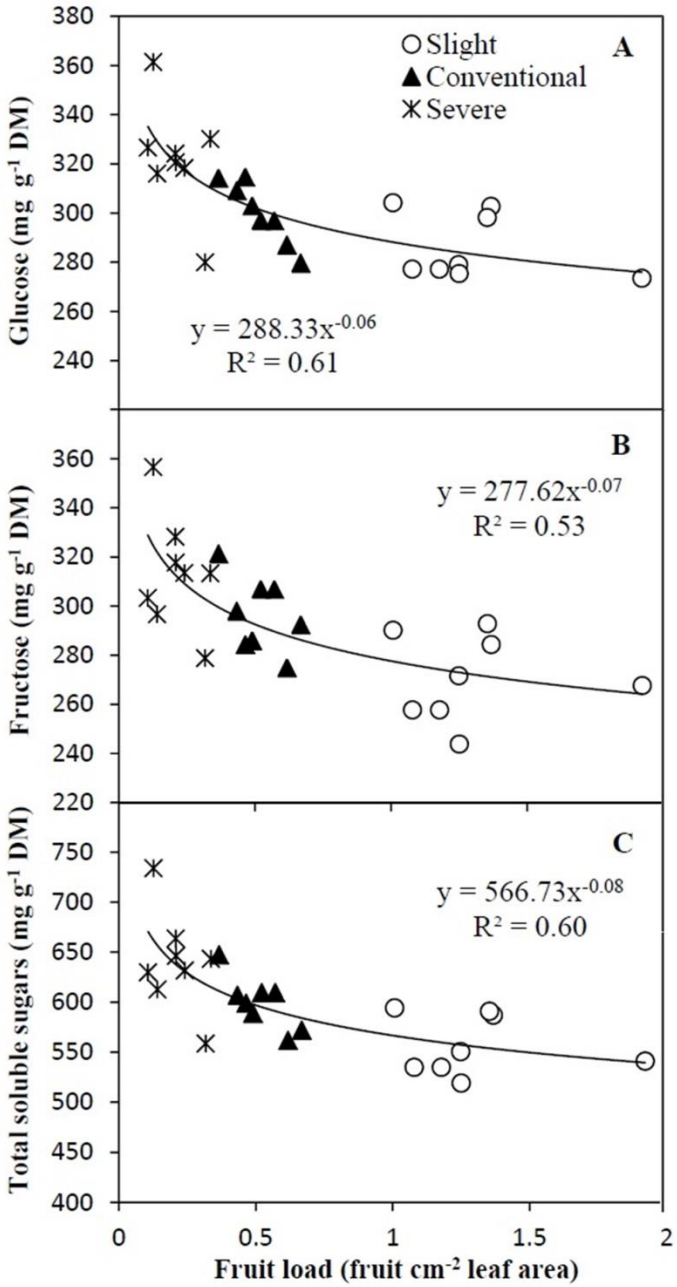

Figure 3. 2 Glucose (A), fructose (B) and total soluble sugars (C) as a function of fruit load for 'Brigitta' highbush blueberry plants subjected to slight, conventional and severe pruning. Mean values of replicates (n= 4), non-linear regression and R^2 value are shown.

3.3.3 Fruit growth

Measurements of fruit diameter reproduced the double-sigmoid pattern of blueberry fruit growth and accounted for the effect of pruning severity on fruit weight gain, herby validating the use of fruit diameter for estimating RGR (Fig. 3.3 A and B).

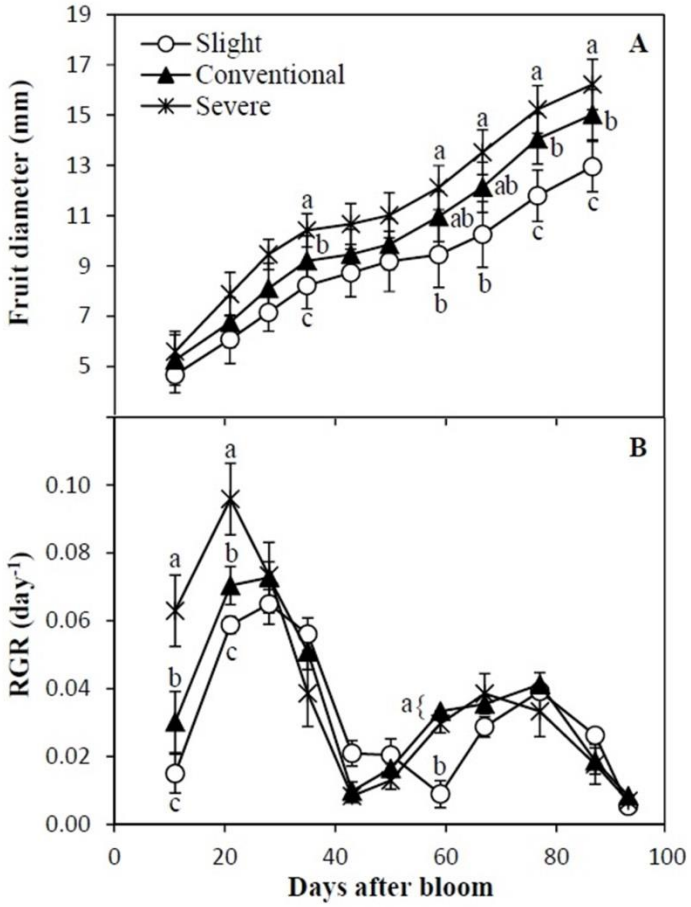

Figure 3.3 Seasonal course of mean fruit diameter (A) and relative fruit growth rate (B) for four-year old 'Brigitta' blueberry plants subjected to slight, conventional and severe pruning. Means (*n*=30) and standard errors (bars) are shown. Letters indicate significant differences (*P*< 0.05) and standard errors (bars) are shown. Letters indicate significant.

As observed in the figure, pruning severity significantly affected RGR at 11, 21 and 59 DAB, with fruits from severely and conventionally pruned plants having higher RGR values than fruits from slightly pruned plants for these dates.

3.3.4 Leaf gas exchange

The photosynthetic light-response curves per pruning treatment were unaffected by the date in which they were performed (67 and 94 DAB, data not shown). The A_n reached light saturation at 700 µmol (photon) m^{-2} s^{-1} (Fig. 3.4).

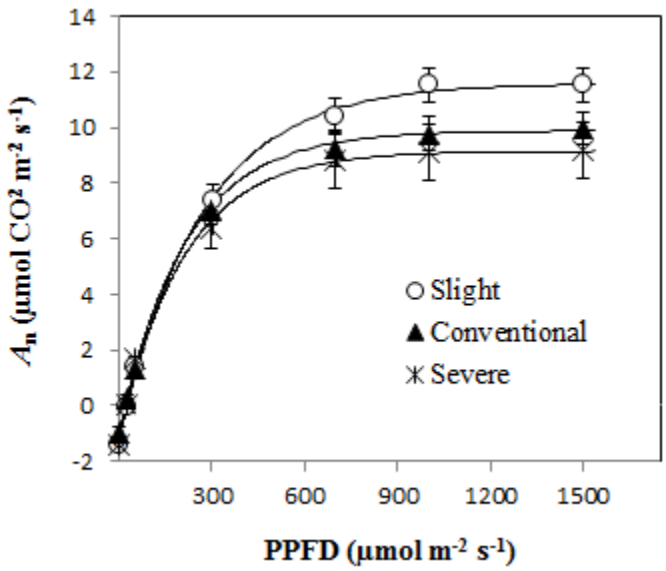

Figure 3.4 Leaf net photosynthesis (A_n) as a function of photosynthetic photon flux density (PPFD) for four-year old highbush blueberry plants cv. 'Brigitta' subjected to slight, conventional and severe pruning. Means for two dates of each treatment (n= 12) and standard deviation (bars) are shown.

The asymptotic model (Eq. 3) was significantly fitted for each photosynthetic light-response curve constructed (R^2> 0.90, P< 0.001 for all empirical coefficients). The A_e^{sat} was significanly affected by pruning treatments, with leaves in the slightly pruned plants having 15% and 24% higher rates than leaves in the conventionally and severely pruned plants, respectively (Table 3.3). The R_d and a_{light} were unaffected by pruning severity (Table 3.3).

Table 3.3 Effect of pruning severity treatments (slight, conventional and severe pruning) on estimated light-saturated photosynthesis (A_e^{sat}), dark respiration (R_d), and quantum use efficiency for CO_2 assimilation (a_{light}) of four-year old 'Brigitta' highbush blueberry plants. Mean of two dates for each treatment (n=12), standard error and letters indicating statistical differences (P< 0.05) are shown.

Pruning treatment	A_e^{sat} **(µmol CO_2 m^{-2} s^{-1})**	R_d **(µmol CO_2 m^{-2} s^{-1})**	a_{light} **(µmol CO_2 µmol^{-1} photon)**
Slight	12.70 (±0.36) a	0.92 (±0.26) a	0.003 (±0.0004) a
Conventional	11.05 (±0.49) b	0.99 (±0.40) a	0.004 (±0.0007) a
Severe	10.24 (±0.62) b	1.09 (±0.51) a	0.004 (±0.001) a

At saturating light, the observed net photosynthesis (A^{sat}) and stomatal conductance (g_s) were correlated through a potential fit with gs accounting for 68% of the variance of A^{sat} (Fig. 5A). The same trend was observed when C_i was plotted against g_s. For this fit, g_s accounted for 67% of the variance of C_i (Fig. 3.5B).

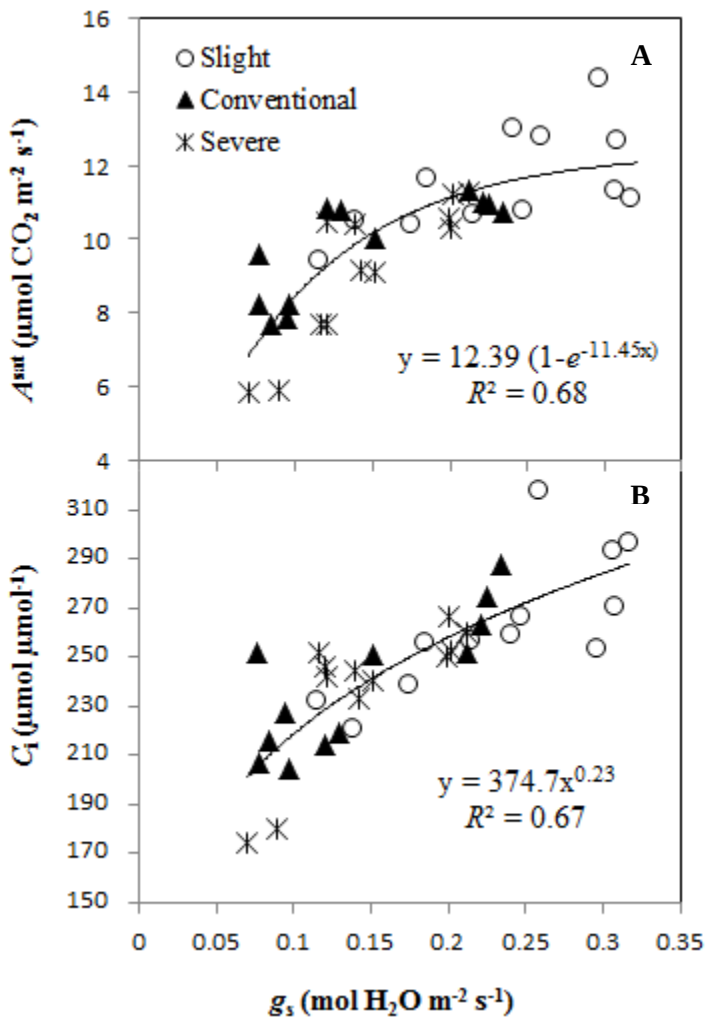

Figure 3.5 Light-saturated photosynthesis rate (A^{sat}) (A) and g_s plotted against internal CO_2 concentration (C_i) (B) as a function of stomatal conductance to water vapour (g_s) for highbush blueberry plants cv. 'Brigitta' subjected to slight, conventional and severe pruning. Mean values (n= 36) measured at saturating PPFD, non-linear regressions and R^2 values are presented.

3.4 Discussion

Pruning is a crucial cultural practice in blueberry production, which provides physical structure to support the current fruit load and sustainability for fruit production in the long-term. Lesser removed wood in 'Brigitta' blueberry plants stimulated a greater yield and berries per plant but reduced whole-canopy leaf area (Table 3.2). The increase in yield and berries per plant was not proportional to the decrease in canopy leaf area when comparing severely and slightly pruned plants. While yield and berries per plant increased close to 2.3 and 4.0 times, respectively, as mean

value of both plant ages, the canopy leaf area decreased by around 40%. This suggests that fruits have higher ability to attract assimilates than leaves and that fruits compete more between them than with shoots, as observed in several species (Gautier et al., 2001; Minchin et al., 2010; El-Boray et al., 2013). Our study showed that 'Brigitta' blueberry fruit weight, %DM and sugars decreased curvilinearly with increasing fruit load (Fig. 3.1 and 3.2). This confirms that pruning severity has an impact on important blueberry fruit quality attributes (Retamales and Hancock, 2012) by regulating the ratio between fruit and canopy leaf area. Although an increasing pruning severity enhanced fruit weight, it did not compensate for the loss in yield per plant. This indicates that yield was limited by sink potential when 'Brigitta' blueberry plants were subjected to severe pruning.

According to Pavel and DeJong (1993b), the periods in which fruit growth is limited by carbon supply can be detected by estimating the relative fruit growth rate (RGR). Thus, when fruit growth is not source-limited, no differences in RGR between fruit growing under different pruning severities should appear. Based on blueberry fruit development phases, in which cell division, the development of the embryo and endosperm tissues and cell enlargement occur in parallel with the changes in accumulated fruit size (Godoy et al., 2008) (Fig. 3.3A), the significant effect of pruning treatments on RGR values at 11, 21 and 59 DAB (Fig. 3.3B) suggests that, in the conventional and slight pruning treatments, the cell division phase experienced a source-limitation, which also occured in the cell enlargment phase for the slight pruning treatment. According to Henton et al. (1999), a source-limited period during early fruit growth may be more detrimental to final weight than equivalent limitations during more advanced stages.

In order to meet the high carbon demand excerted by growing fruits and to counterbalance a reduced increment in canopy leaf area, an enhanced photosynthetic rate was observed in leaves of slightly pruned plants (Fig. 3.4, Table 3.3). This indicates that photosynthetic potential of sun exposed leaves from conventionally and severely pruned plants was operating below its maximum potential. The negative impact of low fruit load on leaf photosynthesis is consistent with observation on several fruit crops including fruit-bearing blueberry shoots (Maust et al., 1999).

Because our photosynthetic light-response curves were assessed on leaves selected from fruiting shoots similar in vigour and fruit load, irrespective of treatment, we can suggest that a high light-saturated photosynthesis was driven by an enhanced assimilate export from these shoots for supplying carbon to the rest of the plant.

The lack of any significant effect of treatments on R_d indicates that lower photosynthesis rates were not influenced by higher substrate consumption (Table 3.3). Similar results were observed in mango leaves by Urban et al. (2004).The finding that pruning treatment did not affect light-response curves at PPFD below 700 µmol (photon) m^{-2} s^{-1} (Fig. 3.4) and a_{light} (Table 3.3), indicates that the utilization of excitation energy is matched by a similar carbon metabolism rate when moderate to low light intensities are experienced. Although light flux density through the plant canopy was not measured in this study, our leaf area results suggest that an important number of leaves experienced moderate to low irradiances, hence, their photosynthesis could be potentially limited by light rather than by fruit assimilate demand. Given that changes in a_{light} have been also associated with proportional changes in *Fv/Fm* related to photo-inhibition of PSII (Duan et al., 2008), lower photosynthesis rates found in conventionally and severely pruned plants were apparently not related with this phenomenon.

Depressed A^{sat} has been also related with: i) decreasing g_s associated changes in C_i, ii) accumulation of soluble sugars in leaves associated to a decrease in electron transport rate, iii) lower nitrogen content in leaves, iv) alterations in any gas exchange component such as an increase in R_d (Urban et al., 2004). We found that measured A^{sat} and g_s were tightly correlated through a curvilinear relationship (Fig. 3.5A), which reflects a proportionally larger increase in g_s than in A^{sat} with increasing fruit load. On the other hand, this response suggests that the co-regulation of photosynthesis and transpiration was not affected by pruning treatments. Similar results were found for coffee trees by DaMatta et al., (2008) and Franck et al., (2006). Decreased g_s resulted in lower C_i in leaves, indicating that a limiting CO_2 concentration in the stomata could be associated with the loss of CO_2 fixation capacity when fruit load steadily decreased as result of a decreasing pruning

intensity (Fig. 3.5B). According to Li et al. (2007), Nebauer et al. (2011), and Urban et al. (2004), decreased A^{sat} was not attributable to a g_s-associated decrease in C_i, when photosynthesis was down-regulated by end-products. Although, end-products of photosynthesis were not measured in this study, from our results we can speculate that end-product of photosynthesis were possibly not accumulated in 'Brigitta' blueberry leaves as a significant and positive correlation between g_s and C_i occurred. Accordingly, DaMatta et al. (2008), proposed that decreased A^{sat} in defruited coffee trees was independent of carbon metabolism and directly related to lower CO_2 availability coupled with lower g_s.

3.5 Conclusion

This study provides new evidence of the effect of pruning severity on leaf and fruit responses in blueberry plants. While slight pruning might provide the potential for higher yield in 4- and 5-year old orchards under southern Chilean conditions, fruit quality would be hampered: lower berry weight, %DM and sugar content. The variations of these quality attributes were explained by variations in fruit load brought about by pruning treatments. A source-limitation to fruit growth occurred apparently at the initial cell division and initial cell enlargement phases as indicated by decreased fruit RGR. The A^{sat} was enhanced with decreasing pruning severity, whereas R_d and a_{light} were unaffected by pruning method. Measured light saturated photosynthesis and g_s were significantly correlated, which suggests that the co-regulation between photosynthesis and transpiration was not affected by pruning treatments. Decreased g_s resulted in lower C_i in leaves, which indicates that a stomatal mediated limitation for CO_2 concentration in the mesophyll could be associated with the loss of CO_2 fixation capacity when fruit load is decreased by pruning. These outcomes improve our knowledge on the agronomic and physiological factors controlling blueberry yield and fruit quality. An estimation of parameters other than those presently studied, such as those related with carbon metabolism of leaves is the next step to be taken in further pruning studies under field conditions.

CHAPTER IV

Rearrangement of physiological and structural leaf traits by changing source-sink relationship in blueberry (Vaccinium corymbosum L.) leaves

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Photosynthetica DOI: 10.1007/s11099-016-0207-9

4.1 Introduction

The source-sink balance is one of the major determinants of plant growth and metabolism, and its understanding can be useful for predicting the effects of agronomical practices affecting either fruit or leaf (or both) and for their inclusion in simulation models. In fruit crops, it is generally assumed that sink limitation during periods of low carbohydrate demand by fruits (due to either low fruit load or periods of low or absent fruit growth), lead to a down-regulation of photosynthetic activity of source leaves. This has been proposed to be caused by an accumulation of non-structural carbohydrates in leaves as a result of disequilibrium between carbon assimilation and assimilate consumption and translocation (Hendrix and Huber, 1986), which decreases the expression of photosynthetic gene promoters (Koch et al., 1992; Jang and Sheen 1994; Eberhard et al., 2008). However, down-regulation of photosynthesis due to an excess of non-structural carbohydrates is not readily observable in all species (e.g. Nebauer et al., 2011).

The reduction in carbon use and assimilation may lead to structural leaf rearrangements if the plant is not able to develop new sink capacity at the medium time scale (days or weeks). In *Mangifera indica* L. branches subjected to high fruit load, leaf nitrogen concentration was higher than in those subjected to low fruit load between 80-90 days after bloom (Urban et al., 2004), suggesting that proteins of Calvin cycle and thylakoids were adjusted to a given reproductive sink demand. On the other hand, chlorophyll concentration has been found to be higher in leaves of fruiting *Malus* x *domestica* Borkh trees than in non-fruiting trees from about 30 days before harvest (Wünsche et al. 2005). Chlorophyll concentration was also higher in fruiting shoots of *Olea europea* L. than in non-fruiting shoots, which was accompanied to higher leaf mass-to-area ratio (Proietii, 2000). These rearrangements can contribute to restore the balance between carbon assimilation and absorbed light energy and can be viewed as a ‘sort’ of acclimation to changing source–sink relationships, which prevents premature cell death and enables efficient nutrient partitioning (Wingler et al., 2004).

Many studies on fruit crop physiology have been based on a source-sink approach, among them, there are studies dealing with photo-inhibition (Duan et al., 2008), fruit quality (e.g. Famiani et al., 1997) and regulation of photosynthesis (e.g. Nebauer et al., 2011; Iglesias et al., 2002; Layne and Flore, 1995). Concerning to blueberry crop (*Vaccinium corymbosum* L), changes in source-sink balance have mainly focused on vegetative and reproductive responses of plants (e.g. Swain and Darnell, 2002; Strik et al., 2003) and recently in some fruit quality traits (Jorquera-Fontena et al., 2014); however, Maust et al., (1999) reported that a low fruit load induces a reduction of net leaf photosynthesis in a southern blueberry cultivar (hybrids of *V. corymbosum* L. with another species of *Vaccinium*), although they did not evaluate if non-structural carbohydrates mediated this depressed carbon assimilation response. Up to now, little is known about the effect of reproductive sink demand on physiological leaf traits such as light-saturated photosynthesis (A^{sat}), stomatal conductance to water vapour (g_s), dark respiration (R_d) and intrinsic water use efficiency ($WUE_i = A^{sat} / g_s$); and structural leaf traits such as nitrogen (N), carbon (C) and pigments concentration.

Considering that blueberry leaves exhibit different carbon assimilation responses to a changing reproductive sink demand, the aim of this study was evaluate the impact of the source-sink relationship by assessing the effects of fruit load and girdling on physiological and structural leaf traits, elucidating if leaf soluble sugar concentration mediates a depressed photosynthesis response to low reproductive sink demand. In order to reach this aim, fully developed sun exposed blueberry leaves were evaluated during the course of the day under different reproductive sink demands.

4.2 Materials and methods

4.2.1 Experimental site and plant material

The experiment was carried out during the 2010-2011 season on a commercial blueberry cv. Brigitta (*Vaccinium corymbosum* L.) orchard, located in La Araucanía region, Chile (38º29'S 72º 23' W). Four years-old plants were spaced every 0.9 m on north-south oriented rows which were 3 m apart on Andisol. Irrigation and fertilizers were supplied and insects and diseases controlled following commercial standards. The experimental site presents a temperate climate, where rainfall is distributed throughout the year (1200 mm y^{-1}), with the highest precipitation during winter and a moderate dry season of less than four months in summer.

4.2.2 Sink-source manipulation

During the second fruit growth stage (70 days after bloom), 72 sun exposed fruit-bearing shoots from 36 homogenous plants (two branches per plant) were selected for uniformity in vigour and fruit load (1-1.4 fruits per leaf) and randomly assigned to apply four treatments of 18 replicated shoots. The fruit load selected was the typical condition found in the orchard plants. Treatments were applied by removing fruits or leaves and consisted in: non-girdled shoots with one fruit per leaf (NG) and girdled shoots with 10 (10F:L), one (1F:L) and 0.1 (0.1F:L) fruit per leaf. Girdling was applied by removing a 2-cm-wide band of bark in the shoot base. The exposed tissues were protected with pruning seal to avoid drying and fungal infection. To restrict sink demand for assimilates mainly to fruits, immature leaves and the apical and axillary buds were removed after applying treatments. The rest of the plant remained intact; no fruit drop was observed during the course of the trial. The NG treatment was used as a reference for assessing the effects of girdling (as compared to 1F:L; in which the fruit load was the same as in NG).

4.2.3 Gas exchange measurements

During the final stage of rapid fruit growth, the A^{sat}, R_d and g_s were measured three times per day (AM: 08:00–10:40; Noon: 13:00–15:40; and PM: 17:00–19:40), on three cloudless days, 10 days after initiating the treatments (on days 80, 85 and 90 after blooming). Six replicated fruiting shoots per treatment were randomly selected to be used on each day of measurement. Thus, two replicated shoots per time of the day were used. In these shoots, three fully developed sun exposed leaves [mean area of 15.74 cm^{-2} ± 0.53cm^{-2} (SE)] close to developing fruits, were measured and then harvested. Samples were immediately placed in a cooler and taken to a freezer in the proximity of the experimental plot. Thereafter, samples were carried to the laboratory for chemical analysis (see below). An infrared gas analyser (Li-6400, LICOR, Nebraska, USA) connected to a broadleaf chamber and with automatic control of leaf temperature, photosynthetic photon flux density (PPFD) and CO_2 concentration, was used for measurements. We assessed A^{sat} applying a saturating PPFD dose of 1500 μmol (photon) m^{-2} s^{-1} (based on preliminary evaluations of photosynthesis light response curves; data not shown). The g_s was recorded for this condition. In the same leaf of the above mentioned evaluation, R_d at a PPFD of 0 μmol (photon) m^{-2} s^{-1} was also recorded. For samples, the WUE_i ($WUE_i = A^{sat} / g_s$) was calculated. Leaf temperature was set at 20 °C and ambient CO_2 and H_2O vapour concentrations were used during measurements. Weather conditions were similar on each measurement day with a mean day time (from dawn to dusk) PPFD of 1061 ± 76.18 (SD) μmol (photon) m^{-2} s^{-1} [with a midday maximum of 2139.5 μmol (photon) m^{-2} s^{-1} at about 13:30-14:30], air temperature of 19.98 ± 2.30 °C, relative humidity of 59.3 ± 8.92 % and water vapour pressure deficit of 1.03 ± 0.12 kPa Rainfalls were absent during days of measurements. Days before evaluations had similar weather conditions.

4.2.4 Chemical analysis

The leaf SSC was extracted from frozen tissue in 86% v/v ethanol with agitation for 24 h and then centrifuged at 13,000 g for 10 min. The supernatant was depigmented with chloroform in a 1:3 v/v mixture (extract: chloroform). Soluble sugar concentration in supernatant was determined spectrophotometrically by Resorcinol method (Roe, 1934) at 520 nm, using sucrose as standard.

Total N and C of each sample were measured on dry leaves using EuroEA 3000 elemental analyser (EuroVector, Italy), designed for CHNS analysis of organic compounds. Water content of these samples was recorded.

Chlorophylls and carotenoids were extracted in dark from frozen leaf tissue with 96% cold ethanol and spectrophotometrically determined at 665, 649, and 470 nm according to Lichtenthaler and Wellburn (1983).

4.2.5 Statistical analysis

Data obtained on each date of measurement were pooled to evaluate both the effect of treatment and the combined effect of treatment and period of the day via Analysis of Variance (ANOVA) followed by Multiple Comparison of Means (Tukey's test at $P < 0.05$). Data were pooled based on a previous ANOVA, which revealed that date of measurement did not affect the results obtained for each treatment and period of the day. A correlation analysis with a *P*-value adjusted via Holm's method was performed to evaluate the correlation among variables. All data analyses and methods used for fitting models were carried out with R free software through 'R' Commander (Rcmdr version 1.8-3 in R version 2.14.2), and 'lm' and 'nls' functions (R version 2.15.0), respectively.

Results from chemical analysis were expressed on mass basis due to the mass-to-leaf area ratio of the samples was unaffected by treatments [122.45 ± 17 (SE) g DM m^{-2}, n=48, data not

shown], thus the hypothetical changes induced by treatments can be attributed to a change in concentration of the variable.

4.3 Results

4.3.1 Gas exchange and soluble sugar concentration

The treatments significantly affected the daily mean A^{sat} of blueberry cv. 'Brigitta' leaves (Fig. 4.1A). Decreasing fruit load resulted in a 54% lower A^{sat} when comparing 0.1.F:L and 10F:L treatments, whereas the effect of girdling reduced A^{sat} by 35 % when comparing 1F:L with NG. For the NG and 10F:L treatments, the daily mean A^{sat} was statistically similar. From AM to PM, A^{sat} was reduced between 22 and 50%, depending on treatment (Fig. 4.1A). For the 0.1F:L treatment, A^{sat} stabilized to a low value from noon onwards, contrasting with the other treatments where A^{sat} was not significantly reduced at midday.

Daily mean g_s was significantly affected by the treatments (Fig. 4.1B). The greatest effect on g_s was brought about by fruit load, with 10F:L treatment exhibiting g_s values more than three times higher than those observed in 0.1F. On the other hand, girdling reduced g_s by around 50% (1F:L v/s NG). In general, the response of g_s to the treatments was paralleled those of A^{sat} over the day (Fig. 4.1B). The g_s values decreased in the range of 11 to 66% from AM to PM, with 10F:L treatment showing the largest variation and 1F:L the smallest one.

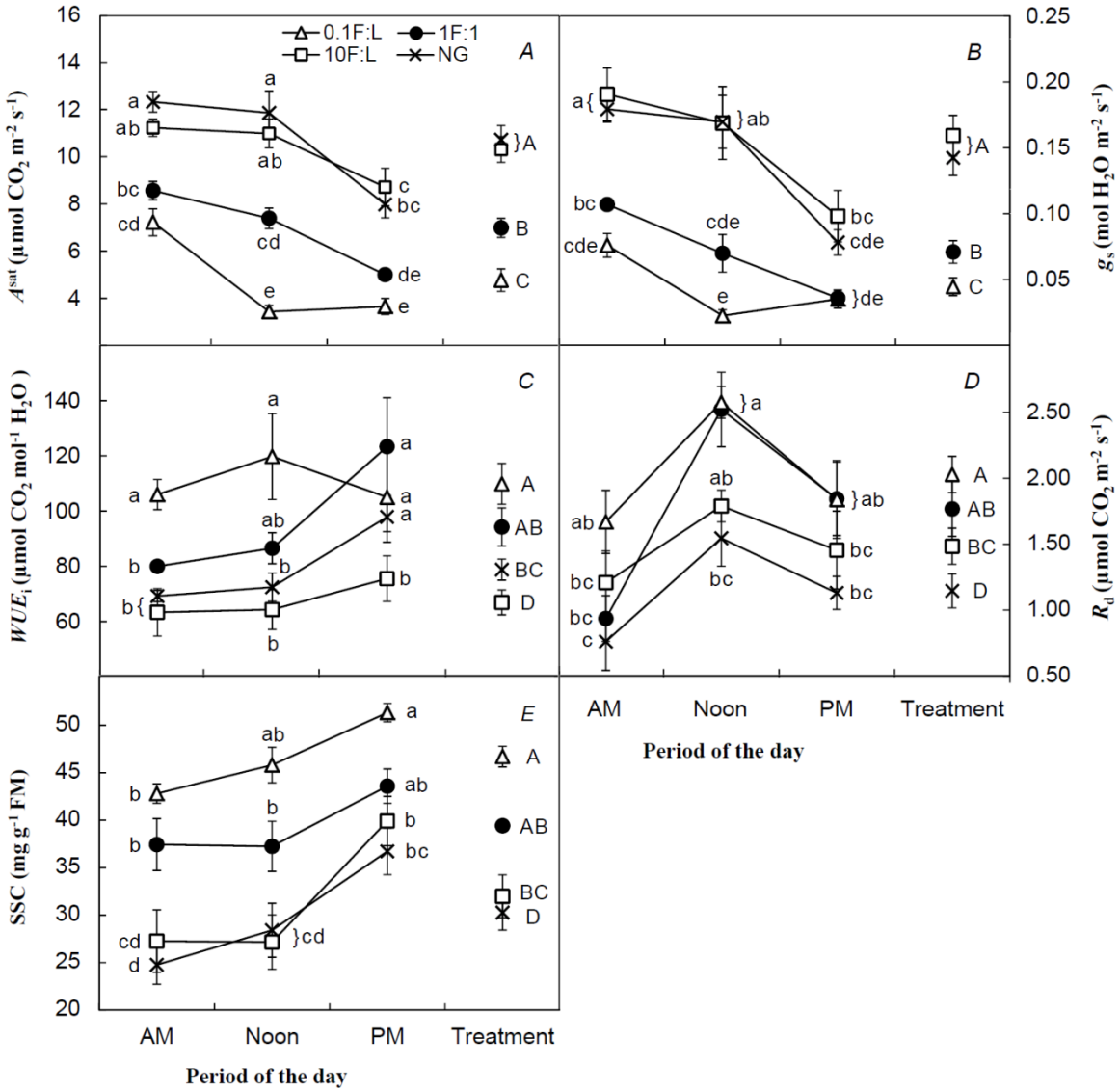

Figure 4.1 Light-saturated photosynthesis (A^{sat}), stomatal conductance to water vapour (g_s), intrinsic water use efficiency (WUE_i), dark respiration (R_d) and soluble sugar concentration (SSC) of 'Brigitta' blueberry leaves from girdled shoots bearing 10 (10F:L), 1 (1F:L) and 0.1 (0.1F:L) fruits per leaf and non-girdled shoots with one fruit per leaf (NG). Mean of treatments (n=18) and period of the day (n=6), standard errors and letters indicating significantly different values at P< 0.05 both for daily mean of treatments (uppercase letters) and the combined effect of treatment and period of the day (lowercase letters), are presented.

For the whole data set, A^{sat} and g_s were closely and positively related, with A^{sat} accounting for 78% of the variance of g_s (Fig. 4.2).

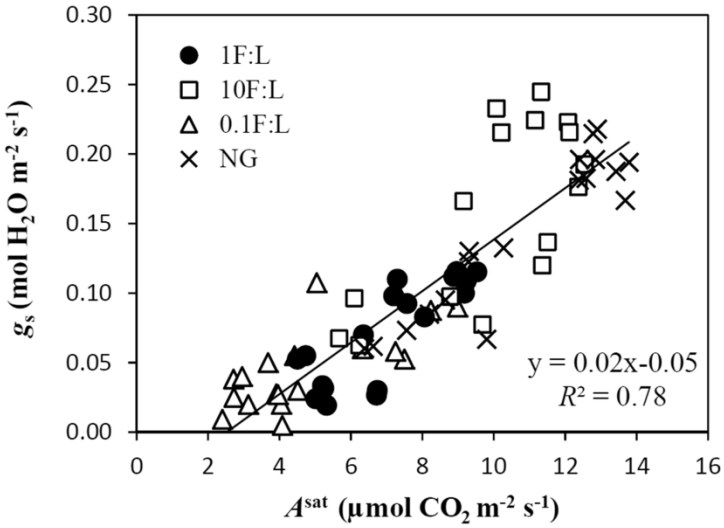

Figure 4.2 Relationship between light-saturated photosynthesis rate (A^{sat}) and stomatal conductance to water vapour (g_s) of 'Brigitta' blueberry leaves from girdled shoots bearing 10 (10F:L), 1 (1F:L) and 0.1 (0.1F:L) fruits per leaf and non-girdled shoots with one fruit per leaf (NG). Non-linear regression (n= 72) and statistical R^2 are presented (linear regressions were not significantly different among treatments). Abbreviation: FM = fresh mass.

The mean intrinsic water use efficiency (WUE_i), as the ratio between A^{sat} and g_s, was affected by treatments (Fig. 4.1C), such that values increased by 83% from 10F:L to 0.1F:L and by 32% from NG to 1F:L. The curse of the day induced to an increased WUE_i values in the 10F:L, 1F:L and NG treatments, reaching values significantly different only in the 1F:L and NG treatments. For the 0.1FL treatment, WUE_i tended to be higher in the noon, although values were no significantly different during the course of the day (Fig. 4.1C).

Daily mean R_d increased with decreasing fruit load and with girdling (Fig. 4.1D). The highest R_d values were observed in the 0.1F:L treatment, which were 77% higher than those of the NG treatment, which exhibited the lowest R_d values. While girdling increased R_d by 54%, decreasing fruit load increased R_d by 37%. Regarding the period of the day, R_d followed a similar pattern in all

treatments: rising between 48 and 128% from morning to noon and decreasing in the afternoon to similar values to those observed in the morning (Fig. 4.1D).

Daily mean SSC of leaves was significantly affected by treatments, as presented in Fig. 4.1E. The SSC was 30% higher in the 0.1FL than in the 10F:L treatment. Girdling resulted in a 23% increase in SSC (1F:L vs. NG), whereas girdled shoots with the highest fruit load (10F:L) exhibited statistically similar values to NG. Diurnal variations of SSC were higher in the extent that sink demand grew (Fig. 4.1E). From AM to PM, SSC of the NG and 10F:L treatments increased around 47%, whereas SSC of the 1F:L and 0.1F:L treatments increased by 8 and 19%, respectively.

For the whole data set, variations of A^{sat} could largely be explained as a negative function of SSC. Thus, SSC accounted for about 78% of the variance of A^{sat} (Fig. 4.3).

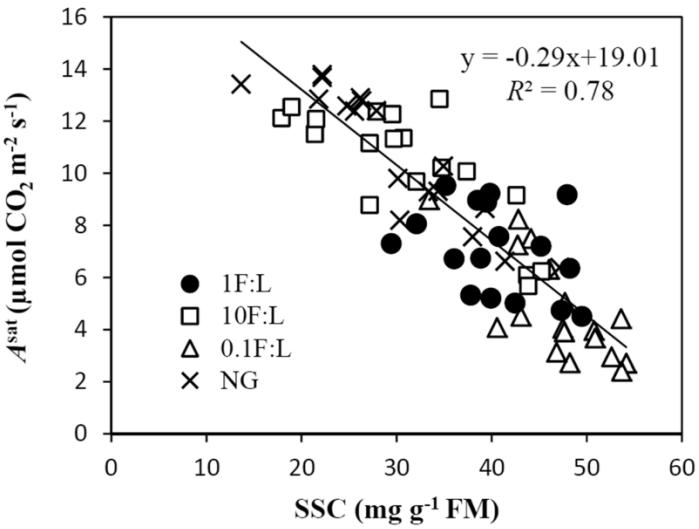

Figure 4.3 Relationship between light-saturated photosynthesis rate (A^{sat}) and soluble sugar concentration (SSC) of 'Brigitta' blueberry leaves from girdled shoots bearing 10 (10F:L), 1 (1F:L) and 0.1 (0.1F:L) fruits per leaf and non-girdled shoots with one fruit per leaf (NG). Linear regression (n= 72) and statistical R^2 are presented. Abbreviation: FM = fresh mass.

4.3.2 Nitrogen and carbon concentration

Daily means of N and C/N ratio were affected by treatments (Table 4.1), whereas the course of the day did not induce changes in these variables (data not shown). As illustrated in Table 4.1, leaf N steadily diminished with decreasing fruit load, reaching a difference of 17% between 0.1F:L

and 10F:L treatments. On the other hand, girdling treatment significantly reduced N by 15% (1F:L vs. NG). The daily mean C remained unchanged by treatments and period of the day, thus the C/N responded to treatments according to the previously described effect of treatments on N (Table 4.1).

Table 4.1 Nitrogen (N) and carbon (C) concentration, and C-to-N ratio (C/N) of 'Brigitta' blueberry leaves from girdled shoots with 10 (10F:L), 1 (1F:L) and 0.1 (0.1F:L) fruits per leaf and non-girdled shoots bearing one fruit per leaf (NG). Mean the treatments (*n*=18), standard errors, effect of treatment and letters indicating significantly different values at $P< 0.05$, are presented. Abbreviation: DM = dry mass.

Treatments	**N ($mg \cdot g^{-1}$DM)**	**C ($mg \cdot g^{-1}$DM)**	**C/N ($mg \cdot mg^{-1}$)**
NG	22.37 (0.38) A	465.39 (16.74) A	20.83 (0.82) A
10F:L	21.87 (0.92) A	451.88 (7.32) A	20.88 (1.10) A
1F:L	18.97 (0.75) B	483.02 (8.38) A	25.61 (0.89) B
0.1F:L	18.12 (0.76) B	458.33 (11.15) A	25.46 (0.96) B
Effect of treatment			
P-value	0.001	0.282	0.001

4.3.3 Photosynthetic pigments

No significant differences among treatments were observed for photosynthetic pigments when comparing periods of the day (data not shown). Photosynthetic pigments as affected by treatments are shown in Table 4.2. The total chlorophyll (Chl_{tot}) was significantly reduced by fruit load, with 0.1F:L treatment having 19% lower Chl_{tot} than the 10F:L treatment. Comparing NG and 1F:L treatments, it was observed that girdling did not induce significant changes in Chl_{tot}. The chlorophyll *a/b* ratio (Chl*a/b*) tended to decrease with sink demand, reaching significant differences only between 10F:L and 1F:L treatments. The daily mean carotenoids (Car) was unaffected by the treatments, but tended to be higher in the 1F:L and 0.1 F:L than in the NG and 10F:L treatments. Daily mean Car/Chl_{tot} ratio was significantly affected by treatments, increasing by 80% from 10F:L to 0.1F:L and by 30% as effect of girdling. In the same line of the other studied variables, the 10F:L and NG treatments showed similar values.

Table 4.2 Photosynthetic pigments of 'Brigitta' blueberry leaves from girdled shoots with 10 (10F:L), 1 (1F:L) and 0.1 (0.1F:L) fruits per leaf and non-girdled shoots bearing one fruit per leaf (NG). Means of treatments (n=18), standard errors, effect of treatment and letters indicating significantly different values at P< 0.05, are presented. Abbreviation: FM = fresh mass.

Treatments	**Chl_{tot}** ($mg \cdot g^{-1}FM$)	**Chl *a/b*** ($mg \cdot g^{-1}FM$)	**Car** ($mg \cdot g^{-1}FM$)	**Car/ Chl_{tot}** ($mg \cdot mg^{-1}$)
NG	1.18 (±0.06) A	1.44 (±0.05) AB	0.11 (±0.007) A	0.09 (±0.005) AC
10F:L	1.19 (±0.04) A	1.28 (±0.07) A	0.09 (±0.014) A	0.07 (±0.011) A
1F:L	1.07 (±0.03) AB	1.57 (±0.06) B	0.13 (±0.011) A	0.12 (±0.008) BC
0.1F:L	0.96 (±0.07) B	1.50 (±0.06) AB	0.13 (±0.009) A	0.13 (±0.008) B
Effect of treatment				
P-value	0.020	0.021	0.069	0.003

4.3.4 Correlation among variables as affected by treatments

The correlation coefficients and their significances are shown in Table 4.3. As C and Cart were unaffected by treatments, these variables were excluded from the correlation analysis. The A^{sat} was positively correlated to g_s and N and negatively correlated to most of the other variables except for Chl_{tot} and Chl *a/b* for which no significant correlation was observed. The g_s showed similar negative and positive correlations for the same variables as A^{sat}, excepting an absence of significant correlation to R_d. The WUE_i correlated positively to SSC; and negatively to Chl_{tot}. The SSC also correlated positively to C/N and Car/Chl_{tot} ratio; and negatively to N. The N was also negatively correlated to R_d, whereas Chl *a/b* ratio was positively correlated to Car/Chl_{tot} ratio.

Table 4.3 Matrix of correlation coefficients among the evaluated variables for the main effect of treatment.

	g_s	WUE_i	R_d	SSC	C/N	N	Chl_{tot}	Chl *a*/*b*	Car/Chl_{tot}
A^{sat}	0.93***	-0.7**	-0.64*	-0.94***	-0.76**	0.72**	0.52	-0.39	-0.63*
g_s		-0.84***	-0.58	-0.86***	-0.75**	0.71**	0.57	-0.40	-0.62
WUE_i			0.53	0.66*	0.56	-0.59	-0.73**	0.31	0.58
R_d				0.56	0.67*	-0.72**	-0.60	0.16	0.44
SSC					0.67*	-0.65*	-0.52	0.34	0.63*
C/N							-0.50	0.26	0.40
N							0.55	-0.19	-0.42
Chl_{tot}								-0.21	-0.60
Chl *a*/*b*									0.80***

Levels of statistical significance are: *P<0.05, **P <0.01 and ***P <0.001.

4.4 Discussion

4.4.1 Effect of treatments on the studied variables

Our results show that, already ten days after applying the source-sink treatments, an adjustment in the measured variables occurred in response to the new levels of carbon demand imposed by the treatments. The reduction in carbon demand brought about by girdling and reduced fruit load induced a negative effect on A^{sat} values (Fig. 4.1A), which were in the range of those reported by Maust et al. (1999), in 'Sharpblue' southern blueberry cultivar under different fruit densities (number of fruits per cm of fruit-bearing branch). For the NG and 10F:L treatments, A^{sat} rates were apparently not or slightly limited by sink feedback, exhibiting similar A^{sat} values to those obtained with light-response curves performed under greater fruit load than tested in the NG treatment (daily mean equal to 11.56 ± 0.4 µmol CO_2 $m^{-2}s^{-1}$, n=21, unpublished data). The similarity of A^{sat} for the NG and 10F:L treatments indicates that organs other than fruits have enough carbon demand to sustain high assimilation rates in the NG treatment (non-girdled shoots bearing one fruit per leaf) and that reproductive shoots apparently export assimilated to the rest of the plant (Fig. 4.1A). This is consistent with the fact that carbon partitioning for vegetative growth is increased by reducing fruit load in several species, including blueberry (Maust et al., 1999). Alternatively, the ability of the fruit, both as carbon consumer and driving force for higher assimilation rates, can be observed in the significant increase of A^{sat} in the 10F:L treatment, where the depressing effect of girdling on A^{sat} was appeased (Fig. 4.1A).

The A^{sat} and g_s were strongly correlated (Fig. 4.2 and Table 4.4), which suggests that the co-regulation of photosynthesis and transpiration was not affected by treatments, agreeing with results found in *M. indica* (Urban et al., 2004) and in *Coffea arabica* L. (Franck et al., 2006). On the other hand, lower g_s was not associated with lower intercellular CO_2 concentration (C_i), demonstrating that the depressing effect on A^{sat} is not attributable to a g_s -associated decrease in C_i (data not shown).

The direct dependency of g_s to A^{sat} (Fig. 4.2) resulted in higher WUE_i when a reduced photosynthesis rate occurred. Similar results were found by Gilbert et al., (2011), in different *Glycine max* (L.) Merr genotypes subjected to mild drought. Our results imply that the amount of carbon gained per unit water used is enhanced when 'Brigitta' blueberry leaves experienced lower reproductive sink demand.

A strong negative correlation between A^{sat} and SSC was observed for the whole data set (Fig. 4.3), which increased when the data were arranged as daily means of the treatments (Table 4.3). This confirms the hypothesis that non-structural carbohydrates are involved in sink feedback down-regulation of photosynthesis in blueberry leaves, as reports for other woody crops (e.g. Franck et al., 2006; Wünsche et al., 2005). Sugar biosynthesis in leaves is likely to have exceeded the rate of export to sinks, leading to accumulation of sugars, which decreased the expression of photosynthetic gene promoters (Koch et al., 1992; Jang and Sheen, 1994; Eberhard et al., 2008).

Daily mean R_d increased in the extent that carbon demand was reduced (Fig. 4.1C). Avery et al. (1979), reported that R_d increased by 32% when sinks were restricted in *M. domestica* trees; but Urban et al. (2004), did not find any significant effect of sink on R_d in *M. indica* leaves. Higher respiration rates have been closely related to both higher substrate availability in leaves and higher photosynthetic rate (Noguchi, 2005). Our results showed that R_d and SSC were not significantly correlated (Table 4.3), which indicates that substrate was not a major limiting factor for respiration. In addition, the negative correlation found between A^{sat} and R_d (Table 4.3) suggests that higher respiratory rates in leaves of blueberry cv. 'Brigitta' were not directly related to carbon gain under our study condition (Table 4.3). Similar observations have been previously reported in *Alocasia macrorrhiza* L. (Noguchi et al., 1997) and *Pisum sativum* L. (Azcon-Bieto et al., 1983). According to Noguchi (2005), high rates of processes that use respiratory products (nutrient export and protein turnover) are involved in higher respiration rates uncoupled with leaf carbohydrate status. Considering that R_d was negatively correlated to N (Table 4.3), it is probable that a high respiration

rate is produced by an increased nitrogen remobilization for developing new sink capacity (Paul and Foyer, 2001).

Under the presently evaluated conditions, N was positively correlated to A^{sat} (Table 4.3), which confirms that carbon and nitrogen balance are highly related and co-regulated in function of sink demand (Thiebus-Kaesberg and Lenz, 1994; Paul and Driscoll, 1997). In fact, the strong correlation of A^{sat} to C/N summarizes this assumption, despite that changes in C/N ratio were driven only by variation in N (Table 4.1).

The Chl_{tot} was stabilized at lower values with decreasing sink demand (Table 4.2). As previously reported by Wünsche et al. (2005), and Nii (1997), a decrease in the chlorophyll content of leaves was stimulated by removal of fruit in *M. domestica* and in *Prunus persica* (L.) Batch trees, respectively. Although Chl_{tot} was not significantly correlated to A^{sat} (P=0.19; Table 4.3), a low chlorophyll concentration reduces the absorption of excess radiant energy by leaves (Niinemets, 2007), diminishing the risk of photo-inhibition under conditions in which carbon use is low. In this way, the increase of both Car and Chl *a/b* ratio associated with decreasing sink demand (Table 4.2), added to high correlation between these variables (Table 4.3), indicates that a low sink demand induced adjustments of the light-harvesting pools with a trend for reducing antenna size accompanied to increasing the carotenoid pool. This probably in the aim of enhancing photo-protection, as it occurs in leaves acclimated to high irradiances (Hallik et al., 2012). Higher levels of protection from excess radiant energy are also associated with increasing relative content of Car with respect to Chl_{tot} (Demmig-Adams, 1990; Goncalves et al., 2005). As observed in Table 4.2, Car/Chl_{tot} ratio was significantly affected by treatments with leaves exhibiting higher ratios when sink demand was reduced. The interpretation of increased Car/Chl_{tot} as a photo-protective mechanism under down-regulated photosynthesis conditions can also explain the negative correlation found between A^{sat} and Car/Chl_{tot} (Table 4.3). On the other hand, the significant and positive correlation between Car/Chl_{tot} and SSC (Table 4.3), confirms the association existing between levels of photo-protection and accumulation of sugars in leaves, as an adaptive response to

a stressing condition (Roitsch, 1999). As photo-protection has a high energy cost (Raven, 2011), the activation of such mechanisms could also explain the higher R_D observed in low sink demand treatments (Fig. 4.1C).

4.4.2 Diurnal dynamics of gas exchange parameters and SSC

Diurnal source leaf metabolism is generally regulated as to maintain a relatively steady carbon supply to meet demand of growing sinks throughout day/night periods (Geiger and Servaites, 1994; Fondy and Geiger, 1982). Regardless treatments, data suggest that a gradual reduction in carbon use by fruits over the course of the day occurred (tentatively the daily rate of carbon demand in sinks slows as response to a circadian rhythm), leading to a gradual accumulation of sugars in leaves in concomitance with a decreasing photosynthesis capacity. In fact, our results showed a close correlation between A^{sat} and SSC when taking the data of all the periods of the day (R= -0.88, P< 0.001, n=72), which confirms the major effect of sugar concentration on photosynthetic performance over the diurnal cycle (Eberhard et al., 2008). The gradual accumulation of sugars in leaves allow time for acclimation and restoration of balance of the leaf metabolism (Geiger and Servaites, 1994) and generating substrate to support the night time growth and maintenance processes in the absence of photosynthesis (Walter and Schurr, 2005; Nozue and Maloof, 2006). Our results showed that sugars accumulated in leaves were consumed during the night (Fig. 4.1E), resulting in the highest A^{sat} rates at AM, regardless of treatment (Fig. 4.1A). Nonetheless, as less substrate was consumed in sink limited treatments during the night period, more carbohydrate for translocation to the developing sinks was available on the subsequent day, which resulted in a down-regulated A^{sat} for the 1F:L and 0.1F:L treatments in the morning.

The A^{sat} of the NG, 10F:L and 1F:L treatments remained unchanged from AM to noon (Fig. 4.1A), indicating that levels of carbon consumption by sinks were enough to avoid an early accumulation of sugars (Fig. 4.1E) and loss of photosynthetic rate. The A^{sat} of the 0.1F:L treatment

was significantly reduced and stabilized at a low value at noon, contrasting with observations in the other treatments (Fig. 4.1A). This lower A^{sat} was not accompanied with a significant increase in SSC (Fig. 4.1E), which suggests that mechanisms other than sugar-sensing may play an important role on A^{sat} of 'Brigitta' blueberry leaves under severely reduced carbon demand. In this sense, an increased content of reactive oxygen species produced by excess absorbed light when low sink demand represses photosynthesis, might have resulted in a photo-damage that overcame repair rates of PSII, resulting in a photo-inhibition of PSII. Although we did not measure chlorophyll *a* fluorescence parameters, leaves from *P. persica* branches with reduced fruit load exhibited photo-oxidative damage when higher irradiances were recorded in field conditions (Duan et al., 2008).

Changes in A^{sat} and g_s were sufficiently co-ordinated as to not result in significant differences in WUE_i from AM to noon, irrespective of treatment. This co-ordinated response was altered in the afternoon with g_s decreasing more than A^{sat}, which tended to increase WUE_i for the NG, 10F:L and 1F:L treatments. Although, vapour pressure deficit was not a factor in this study, an increased stomatal closure in response to a high leaf-to air vapour pressure difference occurred later in the day might explain reductions in g_s (Yong et al., 1997). In contrast, WUE_i in the 0.1F:L treatment tended to increase in the afternoon, indicating that a strong A^{sat} reduction by sugar accumulation in leaves, limited carbon assimilation efficiency per unit of water used in 'Brigitta' blueberry leaves under severely reduced carbon demand.

Regardless of treatment, R_d tended to be higher at noon when leaves were subjected to the highest irradiances and temperature. This observed trend became significant for the 1F:L treatment. In this time of the day, an enhanced up-regulation of alternative oxidase pathway possibly occurred in the 1F:L treatment, which could contribute to increase carbohydrate consumption and to increase the size of sink for reducing power (Bartoli et al., 2005), diminishing, in turn, the loss of photosynthesis and the deleterious effect of light absorption under conditions in which no high photosynthesis rates are demanded.

4.5 Conclusion

The reduction in carbon demand brought about by girdling and reduced fruit load induced a negative effect on A^{sat}, which was mediated and modulated, in the course of the day, by accumulation of sugars in leaves. Changes in A^{sat} were in parallel to g_s, but the relation found between these variables increased WUE_i both under reduced sink demand conditions and in the course of the day. When no high photosynthesis rates were demanded, N and Chl_{tot} were reduced, while Car/Chl_{tot} ratio was increased. These findings suggest that structural photosynthetic proteins were reallocated and, concomitantly, a reduced absorption of excess radiation and higher levels of photo-protection occurred. The R_d did not correlate to A^{sat} neither to SSC, which reveals that higher respiratory rates were not directly related to carbon gain. On the other hand, increasing respiration rates might contribute for the N export, as R_d significantly correlated to N. Our results showed that manipulating the source-sink relationships in blueberry cv. 'Brigitta' led to a rearrangement of physiological and structural leaf traits, which allows adjusting the daily balance between carbon assimilation and absorbed light energy.

CHAPTER V

Analysis of blueberry (Vaccinium corymbosum L.*) fruit growth and sugar concentration using an ecophysiological model*

Emilio Jorquera-Fontena, Michel Génard, Miren Alberdi and Nicolás Franck. Paper in preparation

5.1 Introduction

Blueberry fruit has been classified as a true berry and exhibits three stages of growth (e.g. Coombe, 1976; Darnell et al., 1992; Godoy et al., 2008). These stages have been associated with distinct biological processes: in stage I, a rapid increase of pericarp size follows fertilization due to increasing cell number; in stage II, a slower 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, 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 for fresh fruits, which has become an increasingly important aspect of fruit production (Grechi et al., 2008), and consumer expectation (Jaeger and MacFie 2001; Albornoz et al., 2009).

It is known that blueberry fruit size can be controlled by adjusting the fruit load via pruning (e.g., Seifker and Hancock 1987; Strik et al., 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 during the season. A larger fruit weight in peach (Morandi et al., 2008) and apple (Stopar et al., 2002), is obtained at low fruit loads and is closely related with 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 those of soluble sugars (Darnell et al., 1994). The greater weight and sugar content in blueberry fruit, induced by decreasing flower bud density in blueberry shoots (Maust et al., 1999b), suggest the significance of the osmotic effect on fruit

growth. However, it seems that cell number, not cell size, may be the primary determinant of final blueberry fruit size (Swain and Darnell 2002, Johnson et al., 2011). Although blueberry fruit growth and quality have been studied through the manipulation of fruit load and pruning, the effect of these practices on variables related to water and sugars interacting throughout fruit development have not been studied up to now. Because water and sugar accumulation in the fruit is a result of several linked processes, the development and use of ecophysiological models (process-based model) has been proposed as an important tool for understanding these processes (Génard and Lescourret, 2004; Sadras et al., 2008). Unlike for other fruit, ecophysiological models have not been developed neither adapted to blueberry fruit, which raises an opportunity for increasing the knowledge in this species which is especially relevant considering the increasing market demand for this fruit due to its benefits to human health.

From the hypothesis that fresh mass and sugar concentration of blueberry fruit can be simulated on the basis of biophysical rules, the aim of this study was to adapt the model proposed by Fishman and Génard (1998), for modeling peach fruit growth to blueberry fruit in order to use it for analyzing and simulating seasonal variations of fresh fruit mass and sugar content as affected by fruit load. 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 sugar dynamics. Water relations and fluxes, which are intermediate variables of the model, are also discussed.

5.2 Materials and methods

5.2.1 Model description and features

The model is based on a biophysical representation of fruit growth, originally developed for predicting the period of rapid fruit growth of peach (Fishman and Génard, 1998). The fruit is described as a single homogeneous compartment 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) was here considered as model input, which allowed: 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 of new cell wall and its components; and 2) to calculate the dynamic of transformation of carbon (C) into sugar for simulating sugar concentration throughout the growth period. 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 and Génard, 1998).

5.2.1.1 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), ρ is the fruit surface conductance ($cm \cdot h^{-1}$, permeation coefficient of the fruit surface to water vapour), $\alpha = M_W P^*/RT$, with M_W = 18 $g \cdot mol^{-1}$ being the molecular mass of water, P^* the saturation vapor pressure according to the description of Fishman and Génard (1998), T temperature in Kelvin and R the gas constant (83 $cm^3 \cdot bar \cdot mol^{-1} \cdot K^{-1}$). 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 *et al*. 1974). This allowed defining the water influxes into the fruit (U, $g \cdot d^{-1}$) as follows:

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

where a dimensionless constant of proportionally (a) represents the area of the vascular network per fruit surface area, L is the xylem and phloem hydraulic conductivity ($g \cdot cm^{-2} \cdot bar^{-1} \cdot d^{-1}$) between stem and fruit, and ψ (bar) is the water potential of stem and fruit (subscript s and f, respectively). Because a decreased hydraulic conductivity to water influx into the fruit has been reported to occur as fruit development becomes completed (Mazzeo et al., 2013; Morandi et al., 2010; Dichio et al., 2003), we used curvilinear function for including this process:

$$L = \frac{L_{\max}}{1 + \exp^{(p_1 DAB)}} \tag{5}$$

where $L_{\max}$ is the maximum hydraulic conductivity and p_1 is an empirical parameter defining the seasonal decrease of L.

5.2.1.2 Stem and fruit water potential

An experimental regression between air vapour pressure deficit (*VPD*, bar) and stem water potential (bar) established on two days from dawn to dusk in greenhouse conditions was applied in the model such as:

$$\psi_S = -1.39 VDP^{1.19} \tag{6}$$

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

$$\pi_f = \frac{RT\,\Sigma m}{w} + p_2 \tag{7}$$

where T is temperature in Kelvin and R is the gas constant (83 $cm^3 \cdot bar \cdot mol^{-1} \cdot K^{-1}$), w is fruit water content (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) and their corresponding molecular masses ($g \cdot mol^{-1}$). 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 et al., 1990). In the Eq. 7, p_2 is an additional osmotic pressure (bar), which represents compounds other than soluble sugars and potassium contributing to the osmotic pressure. The p_2 was calculated via model calibration (see section: model parameterization). Potassium (K_{rat}, g) was considered as a fixed mass proportion of fruit dry mass accumulated (M_d), while soluble sugar content was calculated by integrating Eq. 8 proposed by Grechi et al. (2008), which is based on a balance of C flow in the fruit:

$$\frac{dCTS(t)}{dt} = CC_{fruit} \cdot \frac{dM_d(t)}{dt} - K \cdot CTS(t) \tag{8}$$

where $dCTS/dt$ is the rate of total soluble sugar influx ($gC \cdot d^{-1}$), CC_{fruit} is the carbon concentration in the fruit dry mass ($gC \cdot gM_d^{-1}$), dM_d/dt is the growth rate of the dry mass ($g \cdot d^{-1}$), CTS is the carbon content from the sum of different kind of sugars in the fruit (gC), and K is the relative rate of

transformation of carbon present in sugars into compounds other than sugars ($g \cdot g^{-1} \cdot d^{-1}$). Because, the integrated Eq. 8 gives results in carbon units, they were then divided by the carbon content of each sugar found in the fruit to satisfy Eq. 7. This requires defining the seasonal dynamic of each sugar due to their different molecular masses and hence osmotic pressures. Because our experimental data showed that only hexoses were found in the present study (which have the same molecular mass), a global pool of sugars (g) was considered. Based on Grechi et al. (2008) approach, we determined (see section: Model parameterization) that *K* strongly depends both on time elapsed since fruit set (in days after bloom, *DAB*) and fruit relative growth rate (*RGR*):

$$K = r_1 (RGR)^{r_2} \exp^{(r_3 DAB)} \tag{9}$$

where r_1, r_2 and r_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, cm^3 that involves water uptake rate [dV/dt]), cell wall extensibility (φ, bar d^{-1}) and a threshold value (Y, bar) of the fruit turgor pressure above which irreversible expansion occurs:

$$dV/dt = V\varphi(P_f \quad Y) \tag{10}$$

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 and Génard 1998). Assuming water density at 1 g cm^{-3} ($V \approx w$) and combining Equations 1, 2, 4 and 8, fruit turgor pressure was calculated as:

$$P_f = \lfloor aA_f L(\psi_S + \pi_f)T_f + V\varphi Y \rfloor / (aA_f L + V\varphi), \text{ (if } P_f \geq Y) \tag{11}$$

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:

$$P_f = \lfloor aA_f L(\psi_s + \pi_f) - T_f \rfloor / (aA_f L), \text{ (if } 0 \leq P_f < Y) \tag{12}$$

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):

$$M_f(t) = w_0 + \int (U - T_f)dt + M_d(t) \tag{13}$$

where w_0 is the initial water mass in the fruit.

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

$$SC(t) = \frac{CTS(t)}{CC_{su}} \cdot \frac{1}{M_d(t)} \tag{14}$$

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 simulation of other intermediate variables of the system such as osmotic and turgor pressure and water influx and transpiration.

5.2.2 Plant material and experimental sites

Five- and four-year old 'Brigitta' blueberry plants growing in two commercial orchards (sites) located in the Region de La Araucanía, Chile (site 1: 38º29' S 72º 23' W; site 2: 38º58'S 72º47' W), were used to achieve different fruit loads through management of pruning intensity. In both orchards, plants were established at a spacing of 3 × 0.9 m, in north–south oriented rows on an Andisol. Orchards differed in culture system; site 1 was managed under conventional culture, while site 2 under organic culture. In both sites, fertilization and control of pests and diseases were applied according to the locally recommended practices for each culture system. Water applications were applied as needed (between November and March) at a rate of 10 to 20 mm per week.

5.2.3 Treatments and measurements

In two consecutive winters (2009 and 2010) in site 1 and in winter 2010 in site 2, three pruning severities were applied on 48 four- and five-year old blueberry plants cv. 'Brigitta' arranged in a randomized complete block design consisting of four replicated blocks distributed in two orchard rows. Treatments corresponded to: slight, conventional and severe pruning, which were applied according the criteria described by Strik et al. (2003). All plants were first slightly pruned and then fruit buds were counted. Thereafter, conventional and severe pruning were applied and fruit buds per plant were adjusted targeting that conventionally and severely pruned plants had close to 50% and 20% of the fruit bud number present in slightly pruned plants. Treatments were expressed in terms of fruit load as number of fruit buds per plant, where high, medium, and low fruit load corresponded to slight, conventional and severe pruning. An additional treatment was adjusted in site 1 during winter 2009, which consisted in a pruning severity that resulted in a medium low fruit load (Table 5.1).

During productive seasons, measurements of fruit equatorial diameter were periodically performed with a digital calliper in three plants of each block of treatments. From these plants, 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 measured diameters were then used for estimating fruit fresh (M_f) and dry (M_d) masses by applying allometric functions ($M_f = 8.50x^{-4}di^{2.76}$, n= 432, R^2= 0.99, n=432 and $M_d = 3.512x^{-5}di^{3.19}$, R^2= 0.98, n= 220), which were previously developed (data not shown). The obtained results for each season and site combination were used either for estimating model parameters or for its validation (Table 5.1). For treatments, the initial mass of water in the fruit (value used for integrating Eq.13) was estimated from the difference between fresh and dry masses of 30 fruits at the beginning of the measurement period.

Table 5.1 Fruit load treatments applied to blueberry cv. 'Brigitta' growing in different systems of culture and seasons. Mean values (± standard deviation) of reproductive buds, abbreviations of treatments and sites are presented. Bold letters indicate treatment used for model calibration and its internal validation. Other treatments were used for external validation.

Treatment	Season	Site	Fruit buds plant^{-1}
High fruit load (H)	2010-2011	1	664.66±49.72
	2010-2011	2	**600.11 (± 18.72)**
	2009-2010	1	**799.69±11.98**
Medium fruit load (M)	2010-2011	1	**353.83±35.51**
	2010-2011	2	290.70 (± 14.89)
	2009-2010	1	**416.83±38.51**
Medium low fruit load (ML)	2009-2010	1	249.12 (± 17.39)
Low fruit load (L)	2010-2011	1	**146.69±14.43**
	2010-2011	2	**128.31 (± 13.34)**
	2009-2010	1	173.71±19.42

Sun exposed fruit clusters from the remaining three plants per block other than those used for diameter measurements were tagged to periodically collect fruit (averaging 14 days) in order to determine its soluble sugars concentration along the season. Picked fruits were frozen to -80 ºC and then freezed dried. Sugars from ground frozen-dried fruits (50 mg) were extracted in 80% ethanol containing maltose ($3g \cdot L^{-1}$) as internal standard, for 1 h at 60 ºC, and in distilled water under the same conditions. Each extraction was followed by a centrifugation at 13,000 X 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.

Potential stem water potential was measured using a pressure chamber PMS (model 1000, Instrument Co., Corvallis, Ore.), following the recommendations of Hsiao (1990). Measurements were made two days from dawn to dusk on 1-year old branches enclosed at least one hour (h) in plastic bags laminated with aluminium foil.

5.2.4 Model inputs

The model inputs were: 1) curve of fruit dry mass growth and 2) mean daily air temperature and humidity. The estimated fruit dry mass for each season and site combination was fitted using a logistic function with 5 parameters:

$$M_d(t) = \frac{A}{1 + e^{(b - cDAB + dDAB^2 - eDAB^3)}} \tag{15}$$

where *A* is the maximal dry mass (g), *DAB* is time in days after bloom and *b*, *c*, *d*, *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 $2x10^{-16}$.

Daily means of air temperature and relative humidity were collected from meteorological stations (Adcon Telemetry, Klosterneuburg, Austria) located near to each orchard (data not shown). From these data *VDP* was calculated to be included in the stem water potential estimation.

5.2.5 Model parameterization

The parameters obtained from independent measurements were: 1) the permeation coefficient (ρ, Eq. 2), which was calculated as proposed by Gilbert et al. (2005) using 12 untagged fruits per treatment collected in the season 2010-2011 at different development stages; and 2) the coefficients *y* and *z* of the allometric equation for estimating fruit surface area from fresh mass (Eq. 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) empirical parameters of conductivity of the composite membrane for water transport and (L_{max} and p_1, Eq. 5); 2) pool of osmotically active solutes, other than sugars and potassium (p_2, Eq.7); 3) threshold value of turgor

pressure and cell wall extensibility (Y and φ, Eq. 10). 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 p_1 and p_2 were first obtained, since their initial values were presently unknown. Initial values for calibrating parameters ($L_{\max}$,Y and φ) were taken from literature (for details see Fishman and Génard (1998).

Sugars found in fruit corresponded to glucose and fructose, which showed little variations in their proportions during the season (averages of 51% and 49%, respectively). A logistic function with 5 parameters (similar to Eq. 15) was used to fit observed sugar concentration as carbon (carbon of hexoses) in order to calculate the relative rate of sugar transformation (K parameter, Eq. 8). The fitted curves showed R^2 ranging between 0.93 and 0.97 and P-values for coefficients ranging between 0.00317 and $2x10^{-16}$. Thus, K was calculated solving Eq. 6 for every selected treatments ($k(t) = \left[CC_{fruit} \cdot dM_d(t)/dt - dCTS/dt\right]/CTS(t)$, $g \cdot g^{-1} \cdot {}^{-1}$). 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 differentiation of $CTS(t)$ and $M_d(t)$, respectively. Assuming K as a genotype-dependent parameter (Grechi et al. 2008) the obtained $K(t)$ curves were then plotted against potential explicative variables, of which the interaction of relative growth rate of the dry mass (RGR=($dM_d[t]/ dt) M_d[t]$) and time (DAB) obtained the best fit (R^2=0.78).

The parameters taken from the literature were: 1) the constant a of the Eq. 4, at 0.0273 (Fishman and Génard, 1998) and 2) the fruit potassium concentration at 0.006 g·g-1 M_d (Ochmian et al., 2010). Parameters estimation and curve fitting were performed in 'R' software (R version 2.11.1). Derivative procedure was also performed with the routines of the 'R' software (Crawley, 2007).

5.2.6 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.

5.2.7 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:

$$RMSE = \sqrt{1/n_d \Sigma (y_i - y^*)^2} \tag{16}$$

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:

$$RRMSE = \frac{RMSE}{\bar{y}} \tag{17}$$

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

5.3 Results

5.3.1 Model parameters

The values of the obtained parameters (except those taken from literature) are summarized in Table 5.2.

Surface fruit conductance (ρ) was not influenced by varying fruit fresh mass development (R^2= 0.15, n= 46, data not shown) and was therefore considered to be constant (68.33 cm h^{-1}). The

value estimated for ρ was low comparing with those reported for peach by Lescourret et al. (2001), but was comparable with those described by Jones and Higgs (1982) for apple (14.4-54 cm h^{-1}) and Ben-Yehoshua et al. (1985) for oranges (32.7cm h^{-1}). The allometric relationship between fruit fresh mass and fruit area had a R^2 equal to 0.97 (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 5.38 bar (P<0.001) in the middle range of Y values reported for a variety of plant tissues (Green et al., 1971; Green and Cummins, 1974; Bradford and Hsiao, 1982) which range from 1 to 9 bar. The value extensibility of the cell wall, φ = 0.14 bar^{-1} d^{-1} (P<0.001) was relatively 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 smaller than the value reported for *Mangifera indica* L. (4.08 bar^{-1} d^{-1}) by Lechaudel et al. (2007).

Table 5.2 List of parameters obtained by independent experiments and model calibration.

Parameter (± SE)	**Meaning and equation**
ρ = 76.71 (±2) cm h^{-1}	Fruit surface conductance (2).
y = 4.33(±0.002), z = 0.66 (±0.007)	Empirical parameters relating fruit area (cm^2) to fruit mass (g) (3).
L_{max} = 3.47 (±0.76) g cm^{-2} bar^{-1} d^{-1}	Maximal conductivity of the composite membrane for water transport (5).
p_1=0.06 (±0.003)	Empirical parameters indicating rate of change of membrane conductivity (5).
p_2=1.82 (±0.12) bar	Pressure given by osmotically active solutes other than sugar and potassium (7).
r_1 =3.15 (±0.81); r_2 = 0.61 (±0.10); r_3= 0.05 (±0.004)	Empirical parameters used to calculate the relative rate of transformation of sugars (9).
Y = 5.38 (±0.10) bar	Threshold value of hydrostatic pressure needed for growth (10)
φ = 0.14 (±0.02) bar^{-1} d^{-1}	Cell wall extensibility (10).

The maximal hydraulic conductivity of the membrane separating the stem and fruit compartments (L_{max}) was estimated to be equal to 3.47 g cm^{-2} bar^{-1} d^{-1} based on a composite membrane area to fruit area ratio of 0.0273 (Fishman and Génard, 1998). This result is comparable with what reported by Steudle et al. (1993), for maize roots (2.33 g cm^{-2} bar^{-1} d^{-1}) but higher than the reported value by Nobel (1974), for plant membranes (0.48 g cm^{-2} bar^{-1} d^{-1}). Hydraulic conductivity sharply decreased during the first 35 days after bloom (*DAB*) and reached zero around 85 *DAB*, which was close to fruit ripening for all treatments (Fig. 5.1).

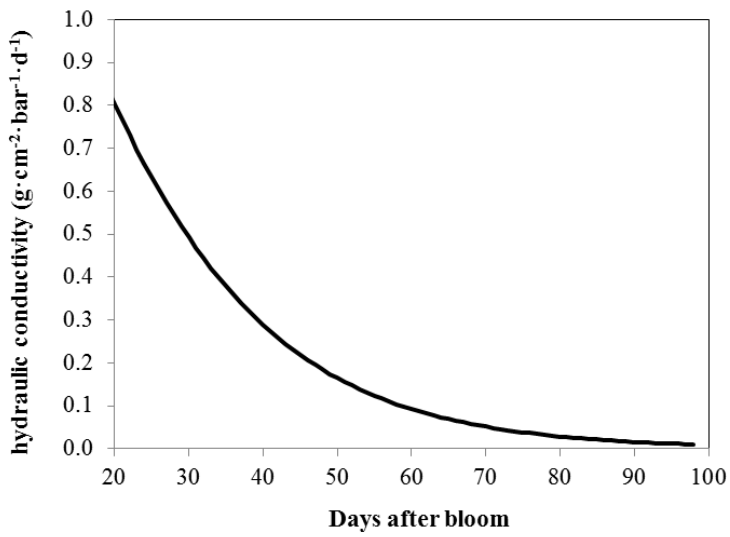

Figure 5.1 Seasonal dynamic of the hydraulic conductivity of the membrane separating stem and fruit.

We solved Eq. 6 to assign values to the relative rates of sugar transformation (Fig. 5.2). As expected, the relative rates of synthesis of compounds other than sugars (e.g. acids, starch, structural carbon, and proteins) tended to decrease along the season, which led to increasing sugar concentration in the fruit. In every case, we found a peak (between 55 to 65 *DAB*) followed by a stable decrease in sugar accumulation rate (Fig. 5.2). The interaction between *RGR* and time (*DAB*), explained 78% of the variability of calculated *K* values, with coefficients highly significant ($P<$ 0.001).

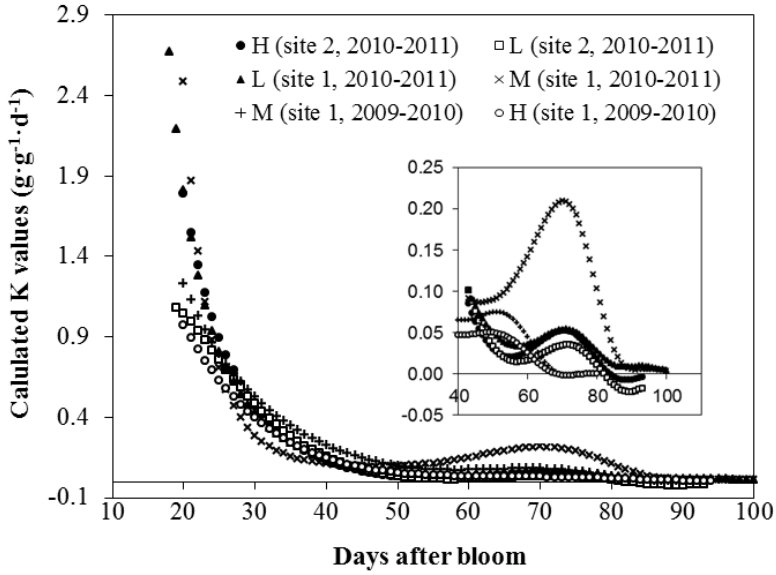

Figure 5.2 Temporal variations of calculated relative rate of transformation of carbon as sugars in the 'Brigitta' blueberry fruit for the synthesis of compounds other than sugars. Initials represent fruit load treatments (H=high fruit load, M=medium fruit load, L=low fruit load). In brackets, sites and productive season are shown.

5.3.2 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 5.3. The double sigmoid pattern of blueberry fruit growth was successfully simulated by the model (Fig. 5.3 A, B and C) with RMSE and RRMSE values for internal validation ranging between 0.05 and 0.12 g and 0.068 and 0.11, respectively.

Sugar concentration was acceptably simulated by the model for all treatments, seasons and culture system (Figure 5.3 D, E and F). The simulations showed values for internal validation ranging between 0.03-0.068 g $g^{-1}M_d$ and between 0.07-0.18 for RMSE and RRMSE, respectively. Nevertheless, marked errors were observed in some treatments: overestimations between 35 and 75 *DAB* (Fig. 5.3D) in M and H of site 1, season 2009-2010 and underestimations at harvest time (Fig. 5.3 E and F).

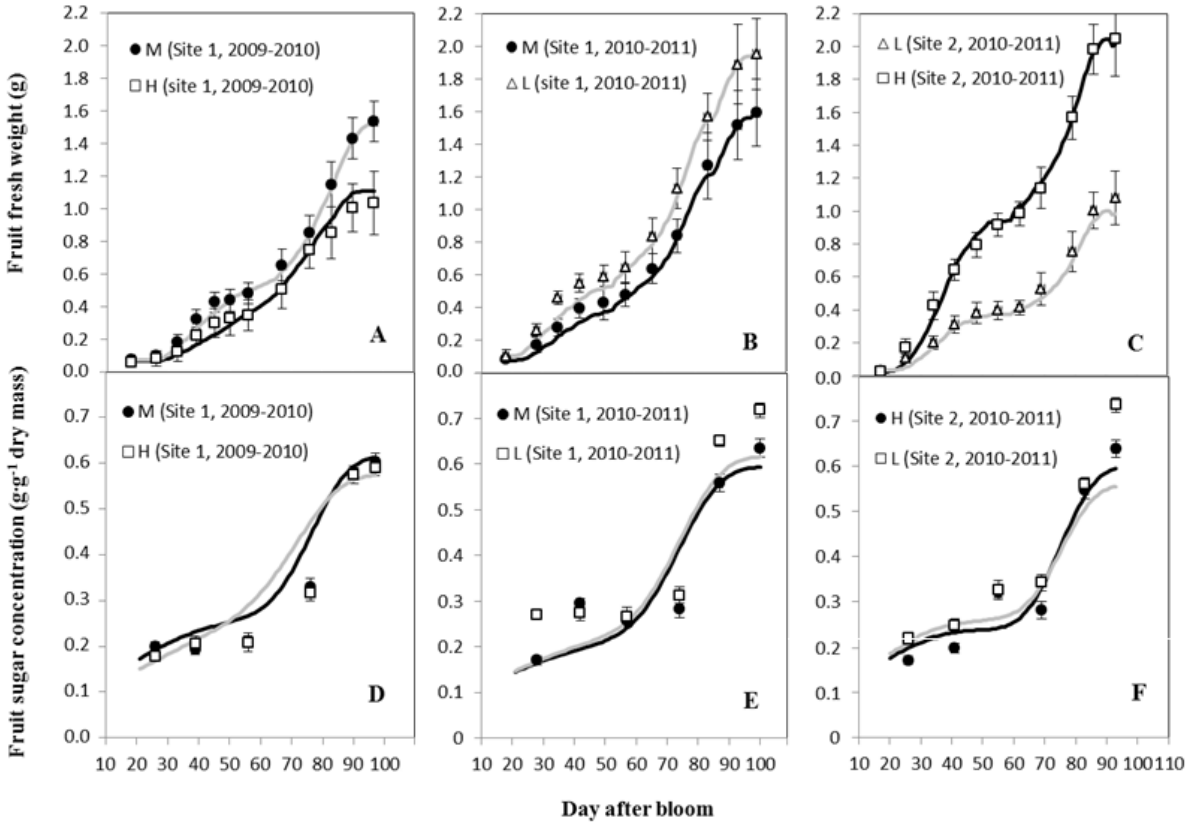

Figure 5.3 Seasonal changes in measured (symbols) and simulated (lines) fruit weight and sugar concentration of 'Brigitta' blueberry fruits as affected by different fruit loads. Initials stand for different fruit loads (H=high fruit load, M=medium fruit load, L=low fruit load). In brackets, sites and productive season are shown. Bars represent standard deviation.

The external validation of fruit fresh mass showed a 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 noticed for the highest values of each treatment which exceed the 1:1 line (Fig. 5.4 A). Values of 0.07 for RMSE and 0.19 for RRMSE were calculated for external validation of simulated sugar concentration (Fig. 5.4 B). In general, the model tended to underestimate for higher values of sugar concentration and overestimate for intermediate values.

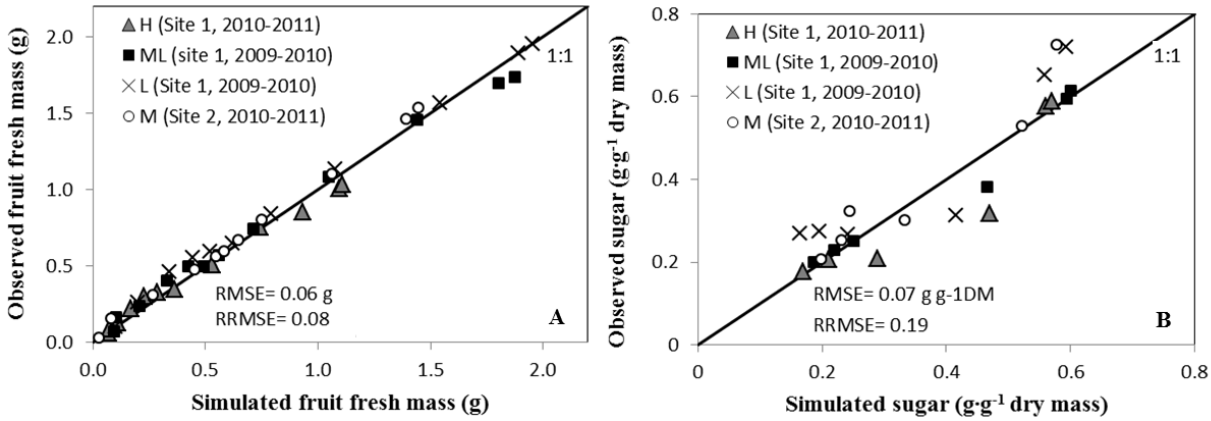

Figure 5.4 Simulated values of fruit fresh weight (A) and simulated values of sugar concentration (B) of blueberry cv. 'Brigitta' plotted against corresponding observed values. Initials indicates high (H), medium (M), medium low (ML) and low (L) fruit load. Sites and productive seasons are shown in brackets. The root mean squared error (RMSE), relative mean squared error (RRMSE) and 1:1 lines are indicated in the figure.

5.3.3 Simulation of water relations and fluxes

Simulated osmotic and turgor pressures and fruit water potential followed a similar pattern along the season, regardless treatments. In general, fruit load did not induce important differences in the simulated values (Fig. 5.5). Due to the close relationship between osmotic pressure and sugar concentration, a growing osmotic pressure was observed along the season. Close to fruit ripening, osmotic pressure tended to a plateau (from about 80 DAB), which was more pronounced in the M and L treatments applied in the site 1 during the season 2010-2011 (Fig. 5.5 B). The turgor pressure increased sharply at the beginning the season and then it was maintained with little variations and with a trend toward decreasing values until that fruit ripening drove to a strong decrease of pressure values (Fig. 5.5 D, E and F). Treatments did not result in important changes on simulated turgor pressure. The increasing differences between osmotic and turgor pressures reduced fruit water potentials for all tested situations (Fig. 5.5 G, H and I).

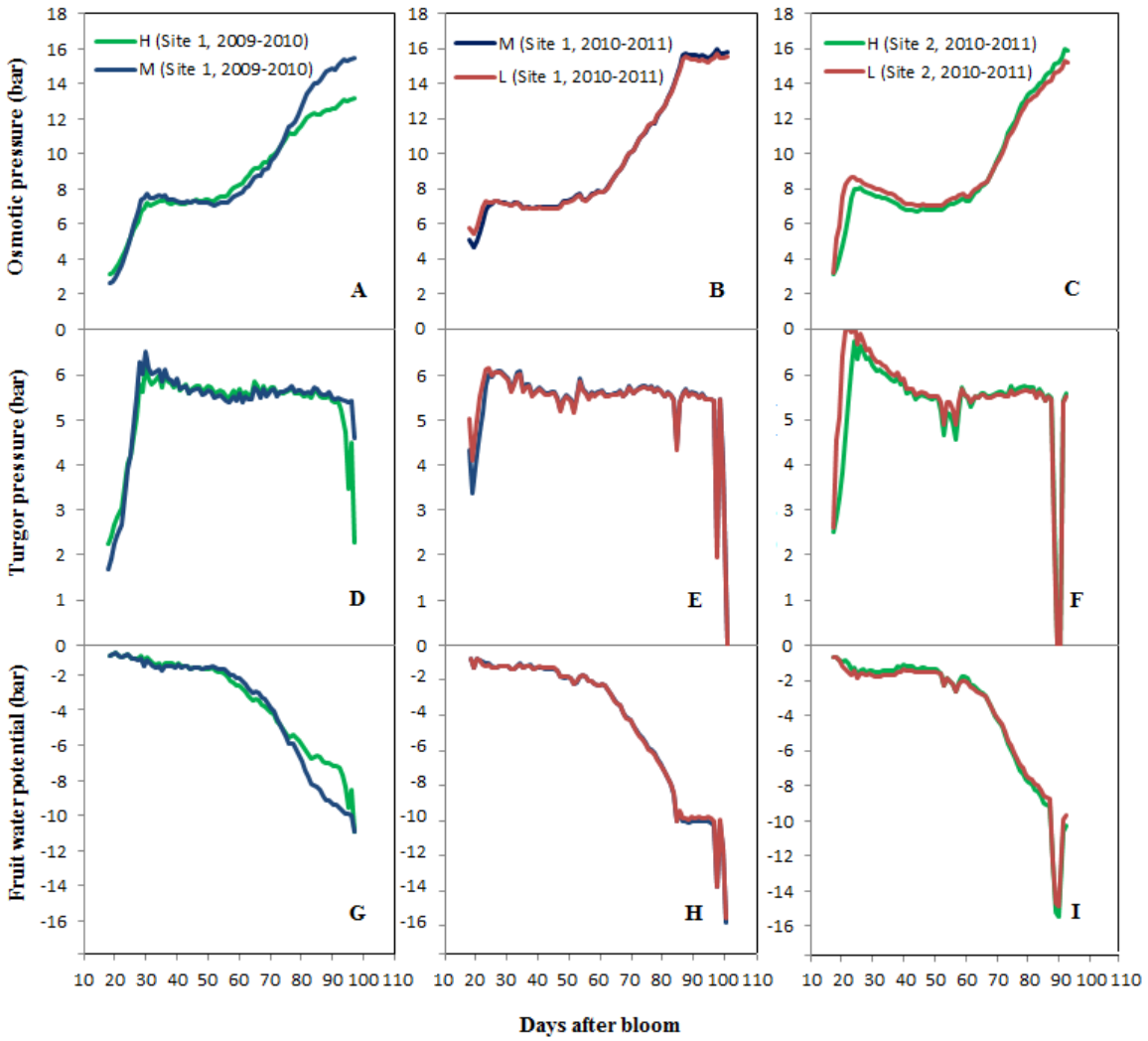

Figure 5.5 Simulation of temporal variations of the osmotic and turgor pressures and resulting water potential for 'Brigitta' blueberry fruits growing under high (H), medium (M) and low (L) fruit load. Sites and productive season are shown in brackets.

Simulated water influx largely changed with treatments (Fig. 5.6 A, B and C). As average value of the season, water influx decreased by about 47% when high fruit load treatments were compared with low fruit load treatments. The gradual increase in air temperature paralleled to a decrease in air humidity along the seasons resulted in increasing transpiration rates (Fig. 5.6 D, E and F). Over the growth periods, the mean transpiration rate decreased by 36% from low to high fruit load. The resulting water balance tended to show two picks, which represent the moment which growth phase changed. At final fruit growth season, water balance showed negative values,

which means that fruit transpiration was higher than water influx, so fruit lost weight (Fig. 5.6 G, H and I).

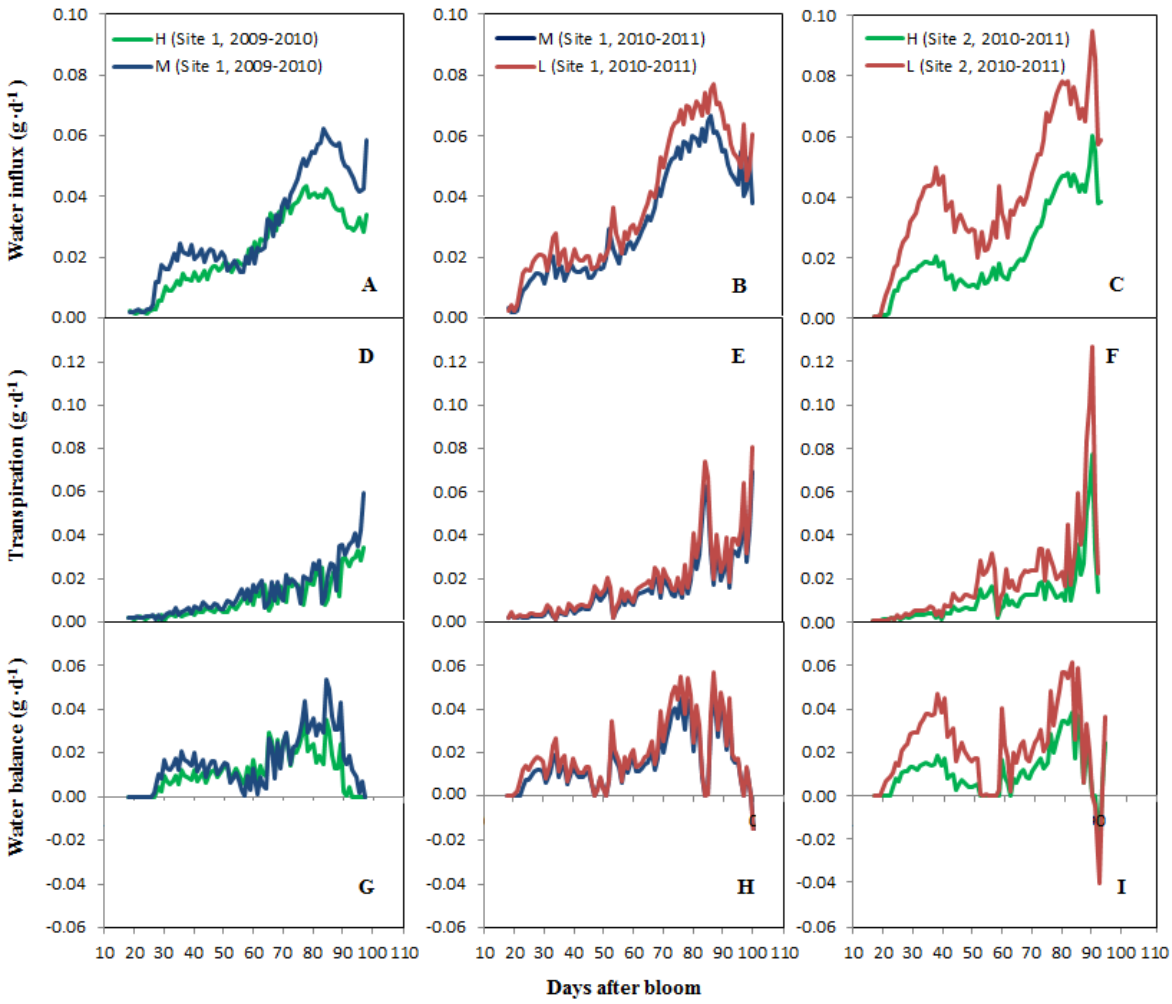

Figure 5.6 Simulation of temporal variations of water influx, transpiration and resulting water balance for 'Brigitta' blueberry fruits growing under high (H), medium (M) and low (L) fruit load. Sites and productive season are shown in brackets.

5.3.4 Parameter sensitivity analysis

The model showed different degrees of sensitivity according to the evaluated parameters and the growth phases (Table 5.3).

Table 5.3 Analysis of model sensitivity for parameters obtained by model calibration and by independent measurements. The variations are expressed as a percentage of the reference value. Variations in fruit fresh mass or sugar concentration exceeding 15% are highlighted with bold characters.

Parameters	**% variation**	**Fresh mass (% variation of g)**			**Sugar concentration (% variation of g g^{-1} dry mass)**		
		Fruit growth phase			Fruit growth phase		
		I	II	III	I	II	III
Allometric:							
Y	20	0.9	4.4	3.7			
	-20	-1.3	2.8	-5.2			
Z	20	-1.6	3.2	0.6			
	-20	1.3	4.3	-0.6			
Water flux:							
Y	20	**-18.5**	**-19.6**	-8.0			
	-20	**16.4**	**20.8**	7.3			
φ	20	1.4	4.1	0.2			
	-20	-2.3	3.2	-0.3			
L_{max}	20	1.1	5.2	7.8			
	-20	-1.6	1.7	-10.8			
p_1	20	-0.5	1.2	**-41.5**			
	-20	0.4	5.6	**24.3**			
ρ	20	-0.2	2.8	-4.6			
	-20	0.2	4.7	4.7			

Table 5.3 (continued)

Parameters	% variation	Fresh mass (% variation)			Sugar concentration (% variation)		
		Fruit growth phase			Fruit growth phase		
		I	II	III	I	II	III
Osmotic:							
From sugars:							
r_1	20	-9.8	-5.7	-4.2	**-15.8**	-14.0	-5.3
	-20	10.9	14.2	4.7	**15.9**	14.2	5.6
r_2	20	13.7	**21.1**	8.1	**20.4**	**23.9**	9.7
	-20	**-16.2**	**-19.4**	-10.1	**-28.7**	**-38.2**	-14.0
r_3	20	5.0	14.8	9.9	8.7	**16.4**	12.9
	-20	-4.5	-7.6	-13.3	-8.4	**-19.0**	**-20.3**
Others:							
p_2	20	5.4	9.3	2.4			
	-20	-5.3	-1.7	-2.3			
K_{rat}	20	3.1	6.3	1.1			
	-20	-3.3	1.1	-1.1			

No larger variations in fresh mass were observed when empirical parameters relating fruit area and fruit fresh mass changed (y and z). The Y induced important alterations in fresh mass mainly in the first and second phase of fruit growth, which were compensated thereafter resulting fruit weight with variations in final weight lower than 10%. In the same way that empirical parameters relating fruit area and fruit fresh mass, changes in φ did not induced important changes in fresh mass whatever growth phase. At harvest, the model was highly sensitive to parameter p_1 (used to estimate the rate of decrease of L_{max}) but was only weakly sensitive to L_{max}. No big changes were observed by variations in ρ. Fresh mass and sugar concentration was also very sensitive to variations of coefficients involved in relative rate of carbon transformation in compounds other than sugar. The r_1 mainly showed great influence on sugar concentration in the first growth period, which was compensated thereafter resulting in variations in sugar concentration lower than 6% at harvest. The r_2 showed the highest influence on fruit growth and sugar concentration, with variations higher than 15% in the first and second fruit growth phase that resulted in fruit weight and sugars higher than 9% at harvest. The r_2 showed to influence mainly in sugar concentration

with final sugars varying over 12%. Variations of parameter p_2 (representing osmotically active solutes other than sugars and potassium), caused important changes when value varied +20% in the second fruit growth phase. These variations were compensated thereafter resulting in variations in final weight lower than 3%. The model was no highly sensitive to changes in K_{rat}.

5.4 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 along 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 other fruits (Bar-Tal et al., 1999; Lechaudel et al., 2007; Liu et al., 2007; Quilot et al., 2005). Our model adaptation to blueberry fruit produces correct simulations, which were validated internally and externally under several contrasting trial conditions with a common set of parameters.

The effect of fruit load on fruit growth and solute content in fruit has been widely studied in fruit crops (e.g. Lechaudel et al., 2005; Souty et al., 1999; Wünsche et al., 2000). In blueberry, the management of fruit load via pruning severity has implications in fruit fresh mass (Swain and Darnell et al., 2002; Strik et al., 2003), which we confirmed and simulated in this study regardless of culture system and years (Fig. 5.3 A, B and C and Fig. 5.4).

The slight differences found in fruit sugar concentration in each tested situation were not adequately reproduced by the model especially at harvest time, with model tending to underestimate the highest values and overestimate the lowest ones, which resulted in a lower goodness-of-fit in comparing with fruit fresh mass (Fig. 5.4). As sugar concentration and osmotic pressures are closely related, the model's inaccuracy resulted in osmotic pressure with scarce differences between fruit load treatments (Fig. 5.5 A, B and C). Nonetheless, when simulation of sugar concentration

enhanced in the L and M treatments from site 1 season 2009-2010 (Fig. 5.3 D, E and F), lower simulated osmotic pressure was observed in fruit growing under high than under medium fruit load in final phase of fruit growth. This leads to low turgor pressure and high fruit water potential (Fig. 5.5 A, D and G) that results in a low increase of water, agreeing with results found for peaches by Fishman and Génard (1998). The inclusion of metabolic activities and environmental variables in sugars modelling might should therefore improve the performance in sugar predictions. In fact, our results suggest that compounds other than sugars translocated into 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 (Fig. 5.2). Such situation has been reported in several fruits (e.g. Coombe 1976, Souleyre et al. 2004). However, the inclusion of other metabolic processes would greatly increase the complexity of the model (Génard et al., 2003).

Our model framework and resulting simulations of water relations, suggest that fruit growth was mainly driven by increases in osmotic potential, as turgor pressure was almost constant during the majority of growth season. This is in agreement with some studies demonstrating a substantial lack of correlation between turgor pressure and growth rate (Schackel et al., 1987 and references therein). As turgor pressure is affected by transpiration rates (Lechaudel et al., 2007; Morandi et al., 2007), it was slightly decreased as fruit transpiration increased by the gradual increase in air temperature paralleled to a decrease in air humidity (Fig. 5.6 D, E and F).

While the pressure components simulated by the model did not widely differ among the evaluated conditions, even when sugars were simulated with a lesser error, water fluxes and their resulting balance showed greater differences (Fig. 5.6). This finding indicates that the relative cell enlargement was not too different among treatments, implying that the differences in fruit mass were mainly induced by cell number. From this, it is hypothesized that source limitation in blueberry cv.'Brigitta' took place during the first stage of fruit growth, when cell division occurs. As reported for 'Herbert' highbush blueberry (Godoy et al., 2008) and rabbiteye blueberry

(*Vaccinium virgatum* Aiton.) genotypes (Johnson et al., 2011), fruit size is primarily facilitated by variation in cell number.

Other osmotically active solutes and their dynamics might be incorporated in our blueberry model to improve its predictive power regarding to pressure model components. In this respect, we estimated the contribution of other osmotically active solutes to osmotic pressure at 1.82 bar (parameter p_2, Table 5.2). 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 would represent the effect of organic acids dominating the osmotically active solutes during these phases in blueberry fruits (Ismail and Kender, 1974).

5.5 Conclusion

The theoretical framework of the present model enabled us to accurately predict the dynamics of blueberry fruit growth and, to a lesser extent, fruit sugar concentration, which represent significant commercial quality traits of the fruits. Our simulations showed that larger fruit weight is mainly commanded by increases in water fluxes rather than pressure differences, which suggest that the number of cells was the main determinant of final fruit weight. The model implementation gave the first estimates of a set of parameters which govern blueberry fruit growth and sugar dynamics. 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. An improvement in the theoretical framework of sugar uptake is the next steps to be taken in order to further develop the model.

CHAPTER VI

General Discussion, Concluding Remarks and Future Directions

6.1 General Discussion and Concluding Remarks

The significant expansion of blueberry production in Chile has been promoted due to suitable marketing opportunities and agro-ecological conditions (Retamales and Hancock, 2012). ODEPA (2013) reported 13,016 ha cropped, with an average production of 102,200 ton and mean yield of 7.8 ton ha^{-1}; however, the projected expansion of this crop may lead to falling fruit prices due to increases export supply. In this probable context, added to the regulation of importer countries, especially in Europe and USA, growers will have to adapt their technical choices to the present concerns about fruit quality.

In Chile, blueberry orchards are mainly oriented to exportation market and cultivated under a high input condition, where diseases are fully controlled and irrigation and fertilizer supply is not a limiting factor. In these conditions, most blueberry cultivars tend to be very productive as a result of a high proportion of photo-assimilates allocated to fruit buds formation in the previous season. This can result in negative implications on the annual outcome of this crop such as imbalance between yield and fruit quality and lower biomass gain to support the following production cycle. In fruit crops, these imbalances have been studied in terms of sink–source relationships (SSR), an interaction between carbon supply and demand by source and sink organs, as reviewed in Chapter II. Studies of SSR have been largely empirical in nature, but these have allowed predictions to be made of the consequences of change to either the supply or demand for photosynthates in several plants (Zamski and Schaffer, 1996). The SSR approach has been studied at different work scales in several fruit crop (Duan et al., 2008; Génard et al., 2009; Nebauer et al., 2011; Iglesias et al., 2002). However, there are not many reports dealing with the effects of SSR in blueberry (Swain and Darnell 2002, Strik et al. 2003), despite that SSR of plants is annually modified via winter pruning management, which regulates fruit load and plant architecture. On the other hand, reports involving to the physiological effects of SSR on blueberry leaf traits are also scarce (Maust et al., 1999a), whereas ecophysiological models have not been used for interpreting and comparing the

quantitative effect of modifying SSR on fruit growth quality despite they are a powerful tool to understand the key-processes involved in the control of fruit growth and sugar accumulation along the production season (Génard and Lescourret, 2004; Génard et al., 2007).

In this thesis, it was investigated the effect of SSR on vegetative and productive responses, fruit quality and physiological and structural leaf traits of field-grown blueberry (*Vaccinium corymbosum* L.) cultivar 'Brigitta'. Two work scales were used to study the effect of varying SSR: i) whole-plant scale through manipulating pruning, and ii) fruiting shoot scale through fruit load adjustment and girdling. The first case is because the shrub is the key level in which most variations in plant performance occur and it is the target of most technical interventions; and the second one, is because fruiting shoot has been postulated as a unit for SSR studies, in which source-sink ratios can be easily obtained (Myers et al., 1999; Iglesias et al., 2002; Urban et al., 2004; Franck et al., 2006). At fruiting shoot scale, girdling was applied to isolate this unit of the buffer capacity of the rest of the plant, regarding carbon balance (Intrigliolo et al., 2009 and references therein). Girdling creates a closed-system environment for carbon metabolism and transport by interrupting the movement of assimilates through the phloem (Roper and Williams, 1989; Li et al., 2003). Besides, it implements an ecophysiological model (process-based model) in order to predict fruit growth and sugar concentration as affected by SSR.

Results supported the hypothesis that pruning severity affects whole plant source-sink relationship in a field-grown highbush blueberry cultivar, inducing either source limitation to fruit growth and quality or sink limitation to leaf carbon assimilation and yield. At fruit-bearing shoot scale, leaf carbon assimilation was steadily reduced when sink limitation increased, with leaf sugar concentration appearing as the driving force behind this effect. When high photosynthesis rates were no demanded, we can speculate that the gradual accumulation of sugars in leaves allow time for acclimation and restoration of daily balance between carbon assimilation and absorbed light energy, as a rearrangement of leaf nitrogen and photosynthetic pigments content occurred.

Our results also underline the significance of pruning practice as determining factor for blueberry yield and fruit quality and can be useful for agro-technical management of pruning.

6.1.1 Yield, fruit growth and quality as response to changing SSR

As viewed in Chapter III, the SSR effect was evaluated by manipulating pruning intensities. Slight pruning increased yield and decreased whole-canopy leaf area, contrasting with what occurred under severe pruning. Intermediate values were found in conventionally pruned plants. As result, varying fruit loads per plant (as fruit allocated to unit of leaf area) were observed. Higher fruit load increased sink demand by higher number of fruits and decreased carbon supply by lower leaf area, which hampered final fruit quality: lower berry weight, %DM and sugar content. This shows that fruit quality was limited by source supply. On the other hand, a high fruit load resulting from slightly pruned plants might provide the potential for higher yield of ‘Brigitta’ blueberry in 4- and 5-year old orchards under southern Chilean conditions. Conversely, an increasing pruning severity enhanced fruit weight, although it did not compensate for the loss in yield per plant. This indicates that yield was limited by sink potential, e.i mean fruit weight approaches to its potential growth, when 'Brigitta' blueberry plants were subjected to severe pruning.

The source limitation to berry weight was produced early in the first and third fruit growth phases, when cell division and cell enlargement occurs, respectively, as relative fruit growth rate decreased. This result was partially confirmed in Chapter V via simulation of water accumulation in fruit. Here, it was shown that the differences in fruit mass as function of fruit load were mainly induced by a source limitation in the first period of fruit growth, agreeing with Godoy et al. (2008), and Johnson et al. (2011), who suggested that variations in fruit size and diameter are primarily facilitated by variation in cell number in blueberry fruits.

Chapter V points out that the use of simulation models for interpreting and comparing the quantitative effect of modifying source-sink relationships was a powerful tool that permitted us to

simulate blueberry fruit growth and quality. The model accounted for these variations with a mean error of 8% for fresh mass and 19% for sugar concentration. Intermediate variables related to water fluxes into fruit were also simulated. Among them, highlight those related to fruit water potential, in which sugar accumulation plays a significant role in fruit osmotic regulation and, in turn, in fruit growth. Model also allowed providing the first estimates of a set of parameters which govern water fluxes and uptake. These were estimated by experimental approach and calibration procedure. From experimental approach we estimated empirical parameters relating fruit area to fruit mass (appendix 1), fruit surface conductance (appendix 4), and stem water potential (appendix 5). From calibration, the maximal conductivity of the composite membrane for water transport, threshold value of hydrostatic pressure needed for growth, cell wall extensibility, pressure given by osmotically active solutes other than sugar and potassium were estimated. The relative rate of transformation of sugars into compounds other than sugar was calculated based on carbon relations in fruits.

6.1.2 Physiological and structural leaf traits as response to varying SSR

In Chapter III, light-response curve were constructed on leaves selected from fruit-bearing shoots similar in vigour, length and number of fruit per leaf (1-1.4 fruits per leaf) to test the hypothesis that leaves on these shoots are not autonomous regarding carbon gain and their light-saturated assimilation rates are driven by whole-plant fruit load resulting from pruning. From this curves a mathematical model was fitted to estimate light-saturated photosynthesis (A^{sat}), dark respiration rate (R_d) and the apparent quantum use efficiency (a_{light}). We found that sink limitation to carbon gain occurred when plants were severely pruned with A^{sat} decreasing by about 34% in comparing with values estimated in slightly pruned plants. These results supported the hypothesis, indicating that blueberry shoots were not autonomous regarding carbon gain. Thus, sink capacity of plants subjected to severe pruning was not able to produce a positive effect for increasing A^{sat} in sun leaves. Conversely, an enhanced A^{sat} occurs probably induced by a high assimilate export from

these shoots for supplying carbon to the rest of the plant when high fruit load resulted from slight pruning.

Because, the use of isolated individual shoots has been amply used as unit level for evaluating SSR in fruit crops (e.g. Franck et al., 2006; Intrigliolo et al., 2009; Urban et al., 2004), this approach was used in Chapter IV. Here, we adjusted SSR by mean of fruit load and girdling. Additionally, non-girdled shoots were used as control of girdling. The A^{sat} showed to be down-regulated under low sink demand with sugar concentration in leaves accounting for about 78% of the variance of A^{sat} (Fig. 4.3). When high photosynthesis rates were no demanded, the gradual accumulation of sugars in leaves allow time for acclimation and restoration of daily balance between carbon assimilation and absorbed light energy, as a rearrangement of leaf nitrogen and photosynthetic pigments content occurred.

In this thesis, A^{sat} and stomatic conductance (g_s) were closely related, demonstrating that co-regulation of photosynthesis and transpiration was not affected by treatments, agreeing with results found in *Mangifera indica* L. (Urban et al. 2004) and *Coffea arabica* L. (Franck et al. 2006). However, the relation between internal stomatic CO_2 concentration (C_i) and g_s at saturating light was largely different according the evaluated scale (Fig. 3.4 and appendix 3). While these variables were positively related when leaves were evaluated in shoots similar in vigour and fruit load but growing in plants subjected to different pruning methods (i.e. different fruit load at whole-plant scale), no relation was found when fruit load was adjusted on girdled fruiting shoots. Changes in g_s coupled to changes in C_i indicate that a stomatic limitation to carbon gain can be operating, so lower A^{sat} of leaves from severe pruned plants, can be related to this phenomenon. Accordingly, DaMatta et al. (2008), demostrated that decreased A^{sat} in defruited coffee trees was directly related to lower CO_2 availability coupled with lower g_s and independent of carbon metabolism. Although, end-products of photosynthesis were not measured when different pruning intensities were evaluated, we can speculate that non-structural carbohydrates were possibly not accumulated, as a significant and positive correlation between g_s and C_i occurred. Conversely, decreased A^{sat} was not attributable

to a g_s-associated decrease in C_i, when photosynthesis was down-regulated by sugar concentration in leaves at fruit-bearing shoot scale. Similar results were found by Li et al. (2007), Nebauer et al. (2011), and Urban et al. (2004).

In Chapter III, the apparent quantum use efficiency for CO_2 assimilation (a_{light}) was unaffected by pruning treatment. Given that changes in a_{light} have been also associated with proportional changes in *Fv/Fm* related to photo-inhibition of PSII (Duan et al., 2008), lower photosynthesis rates from conventionally and severely pruned plants were apparently not related with this phenomenon. In relation with photo-protective mechanism, an enhanced Carotenoids-to-Chlorophylls ratio found in the Chapter IV, points towards a higher photo-protected when lower sink demand were adjusted at fruiting shoot scale.

The relation between measured dark respiration rate (R_d) and measured A^{sat} was also different in both systems. No relation between R_d and A^{sat} was found when fruit load differed at whole-plant level, while an inverse and significant relation was found when fruit load differed in girdled fruit-bearing shoots (Table 4.3). Higher R_d has been largely related to higher substrate levels given by a high photosynthesis (Noguchi, 2005 and references therein); however 'Brigitta' blueberry leaves did not show such response. Processes related with nitrogen reallocation and photo-protective mechanisms were speculated to occur when higher R_d occurred under low reproductive sink demand at girdled fruiting shoot scale.

Light-response curves did not differ among pruning treatments below PPFD ≈ 700 µmol (photon) m^{-2} s^{-1} (Fig. 3.3, Chapter III), and a_{light} was unaffected by treatments (Table 3.3). This indicates that the utilization of excitation energy was matched by a similar carbon metabolism rate when moderate to low light intensities were experienced by leaves. Although light flux density through the plant canopy was not measured in this study, our whole-canopy leaf area (Table 3.2) and light intercepted by the canopy (appendix 2) results, suggest that an important number of leaves experienced low irradiances, hence, their photosynthesis could be potentially limited by light rather than assimilate demand by sinks.

6.2 Future directions outlined from this thesis

- From agronomic point of view, future research on SSR in blueberry plants should be extended to include other fruit quality traits depending on plant carbon economy, such as organic acid, which play an important role in fruit taste.
- Considering that blueberry industry in Chile is focused in export for fresh market, which requires the use of hand harvesting, the agronomic research in pruning should be addressed to optimize the picking efficiency, since costs derived from this practice are steadily growing.
- Biochemical mechanisms underlying the leaf responses to sink demand such as chlorophyll fluorescence, concentration of xanthophyll cycle carotenoids, and key parameters of photosynthetic capacity such as the maximal rate of carboxylation and the light-saturated rate of electron transport, must be elucidated in future researcher of source-sink physiology in blueberry plants.
- The key aspect of the interaction between source and sink organs is to elaborate a conceptual model that permits to establish a basis for the implementation of a decision support system in which pruning is the key factor for simulating yield and fruit quality. The ecophysiological models (process-based models) are increasingly expected to include genetic information via genotype-dependent parameters. These parameters could be considered as quantitative traits and submitted to analysis.

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APPENDICES

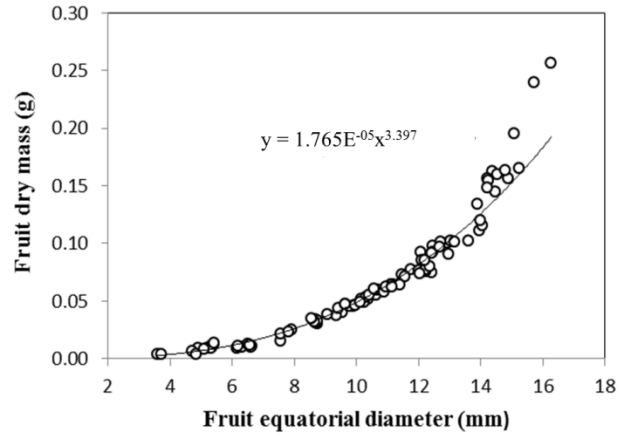
APPENDIX 1

Allometric relations

Relation between fruit equatorial diameter and fruit dry mass used in the Chapter III

Parameter value	Std. Error	t-value	Significance
a = 1.765×10^{-5}	3.565×10^{-6}	4.949	3.14×10^{-6}
b = 3.397	7.742×10^{-2}	43.873	$<2 \times 10^{-16}$

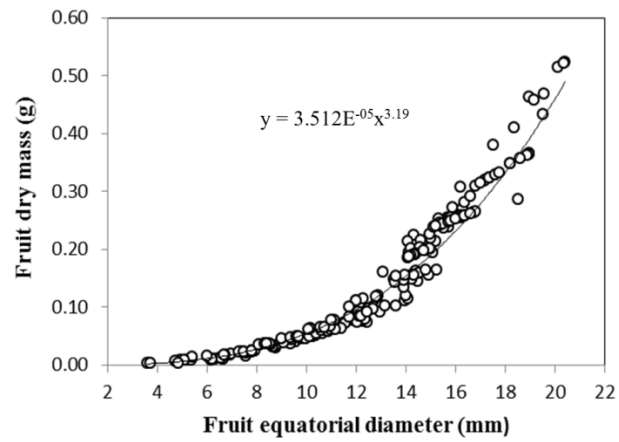
Residual standard error: 0.008885 on 97 degrees of freedom, adjusted R^2= 0.97



Relation between fruit equatorial diameter and fruit dry mass used in the Chapter V

Parameter value	Std. Error	t-value	Significance
a = 3.512×10^{-5}	4.820×10^{-6}	7.288	5.71×10^{-12}
b = 3.19	4.869×10^{-2}	65.508	$<2 \times 10^{-16}$

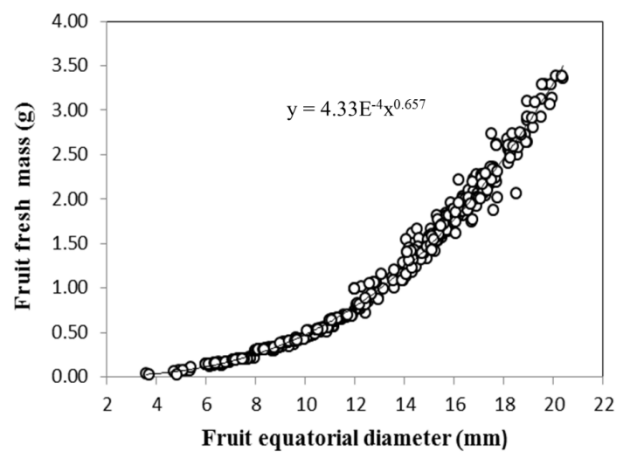
Residual standard error: 0.02031 on 218 degrees of freedom, adjusted R^2= 0.98



Relation between fruit equatorial diameter and fruit fresh mass used in the Chapter V

Parameter value	Std. Error	t-value	Significance
a = 8.501×10^{-4}	0.501×10^{-4}	16.68	$<2 \times 10^{-16}$
b = 2.758	0.0213	129.64	$<2 \times 10^{-16}$

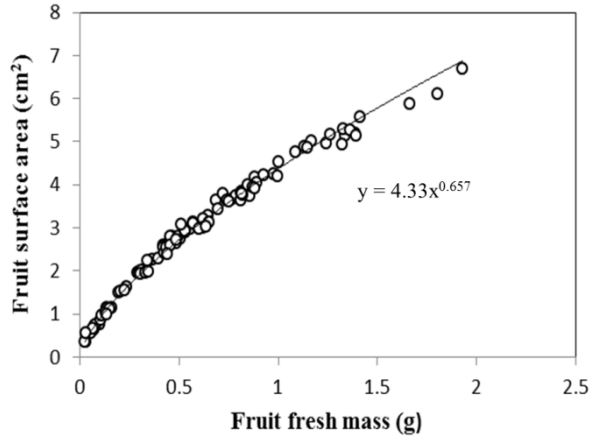
Residual standard error: 0.09414 on 430 degrees of freedom, adjusted R^2= 0.99



Relation between fruit surface* and fruit fresh mass in the Chapter V

Parameter value	Std. Error	t-value	Significance
a = 4.332	0.017	258.24	$<2x10^{-16}$
b = 0.657	0.00688	95.39	$<2x10^{-16}$

Residual standard error: 0.1231 on 97 degrees of freedom, adjusted R^2= 0.99



*Fruit surface was estimated assuming an ellipsoid

APPENDIX 2

Estimation of canopy light interception of blueberry plants cultivar 'Brigitta'

Canopy radiation interception as photosynthetic active radiation (PAR) was periodically measured from 50% bloom to 90% harvested fruit on each block of treatments (exportable fruit). This was conducted using a line quantum sensor (model LI-191SA; LI-COR Inc., Lincoln, Neb). Readings were carried out at solar noon (between 12:30-14:00 hrs, considering ±30° from azimuth as maximum) avoiding cloudy days. The sensor was positioned beneath the plant canopy at ground and above-canopy level to measure both non-intercepted and incoming PAR, respectively. At ground level, readings were performed on transects located perpendicular to the rows, which were 9 cm apart up to reach the area allocated for three plant (2,7m^2), leaving out the edge plants. At above-canopy level, readings were made at the beginning and at the end of the ground level measurement to consider the temporal variation of PAR during measurement. Mean of readings was calculated to estimate the fraction of intercepted PAR (*fPARi*) using Equation 1.

$$fPARi = 1 - \frac{PARni}{PARinc} \quad (1)$$

where *PARni* is non-intercepted PAR, and *PARinc* is incoming PAR. In order to estimate light intercepted by the annual growth, PAR intercepted at 50% bloom was discounted of the subsequent readings performed along the evaluated period.

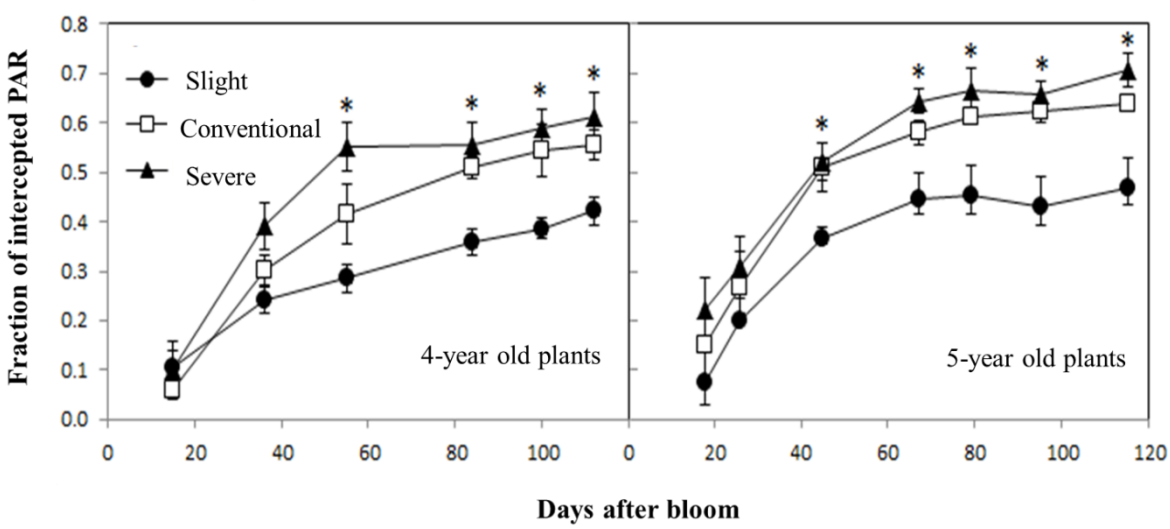


Figure 1 Seasonal variation of intercepted PAR fraction of highbush blueberry cv. 'Brigitta' subjected to different pruning severities. Means and standard deviation (bars) are showed. Asterisks indicate significant differences ($P < 0.05$) among pruning treatments (see Chapter III) at each reading date.

APPENDIX 3.

Relation between stomatic conductance and internal stomatic CO_2 concentration

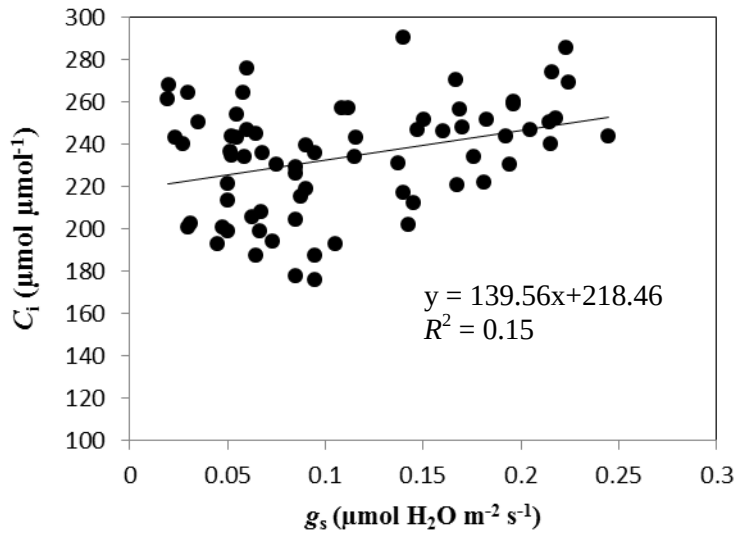


Figure 1. Stomatal conductance (g_s) plotted against internal CO_2 concentration (C_i) for highbush blueberry leaves cv. 'Brigitta' subjected to differennt leaf-to-frui ratios on girdled shoots. Values (n=72) at saturating PPFD [≈ 1500 µmol (photon) m^{-2} s^{-1}], linear regression and and adjusted R^2 are shown.

APPENDIX 4

Fruit surface conductance

Permeation coefficient ρ of water vapour through the fruit surface (cmh^{-1}) can be estimated by rate of water loss from hanging fruit (Fishman and Génard, 1998) as shown Equation 1:

$$\rho = TfRT/(Af \cdot M_w \cdot VPD) \quad (1)$$

where Tf is the rate of water loss per unit of time as a result of transpiration ($g\ h^{-1}$), R is the gas constant ($83\ cm^3\ bar\ mol^{-1}$), T is absolute temperature (K), Af is the fruit surface area (cm^2), M_w is the molecular mass of the water ($g\ mol^{-1}$) and VDP is vapour pressure deficit.

Freshly harvested blueberries were placed in a room with controlled temperature and humidity and weighed 8 times at intervals of one hour after the fruit surface area estimation. Surface conductance was plotted as a function of fresh fruit weight (Lescourret et al., 2001) in order to incorporate in the model (Chapter V). If there is no-relation between these variables, surface conductance will be constant.

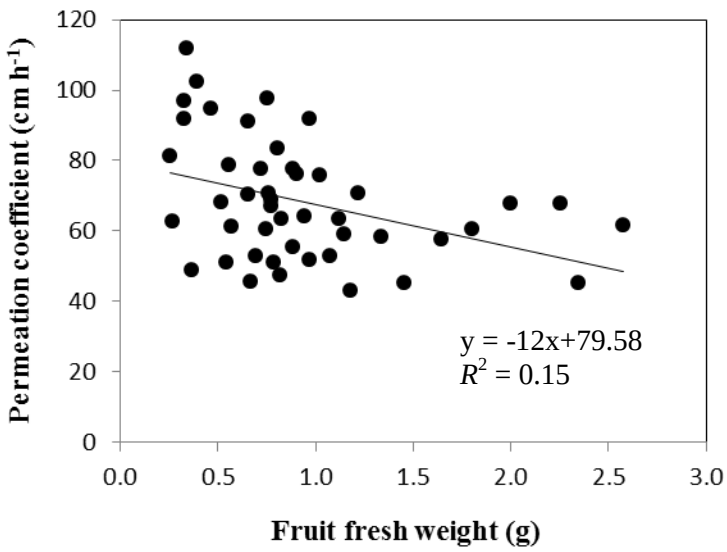


Figure 1 Permeation coefficient plotted against fresh mass of blueberry fruits cultivar 'Brigitta' (n=46). Linear regression and adjusted R^2 are shown.

APPENDIX 5

Stem water potential

For the Chapter V, stem water potential was measured using a pressure chamber PMS (model 1000, Instrument Co., Corvallis, Ore.), under greenhouse conditions, following the recommendations of Hsiao (1990). Measurements were made two days from dawn to dusk on 1-year old branches enclosed at least one hour (h) in plastic bags laminated with aluminum foil. In the greenhouse, temperature and humidity were recorded in order to correlate measured stem water potential to vapour deficit pressure (*VDP*) (McCutchan and Shackel, 1992; Liu et al., 2007).

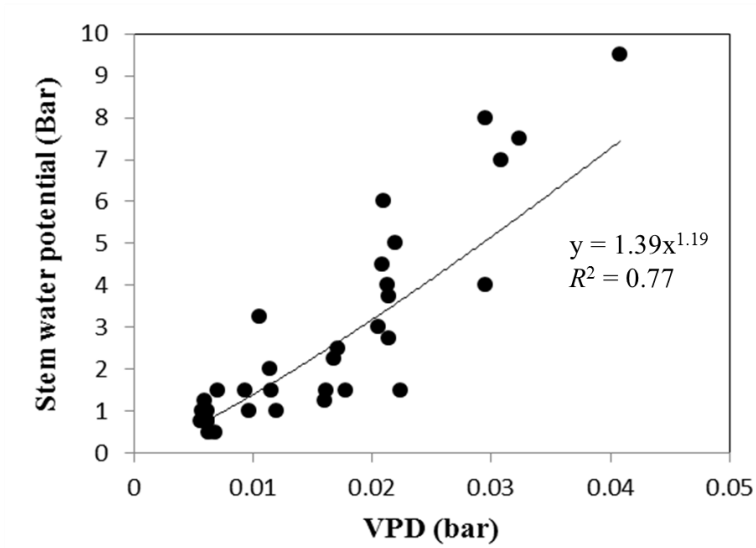


Figure 1 Stem water potential plotted against vapour pressure deficit (*n*= 35). Non-linear regression and adjusted R^2 are shown.

From equation obtained by correlating the measured stem water potential and VDP, we estimated the daily means stem water potentials (ψ) for sites and seasons in which trials were performed. Using daily means of temperature and humidity obtained from meteorological stations close to the trials (Fig. 2), the daily VDP was calculated according to Allen et al. (2006) (Fig. 3).

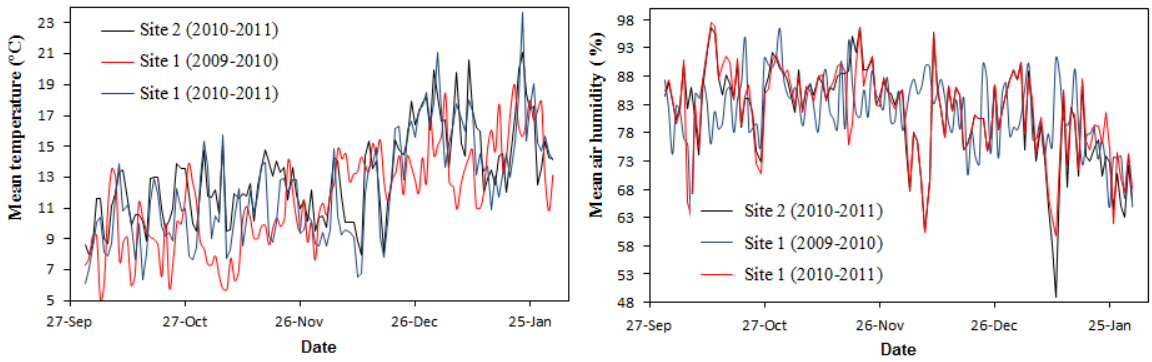


Figure 2 Daily mean temperature (°C) and relative air humidity (%) for the seasons and years in which trials were performed. Site 1: 38º29' S 72º 23' W and Site 2: 38º58'S 72º47' W. In brackets, growth season is shown.

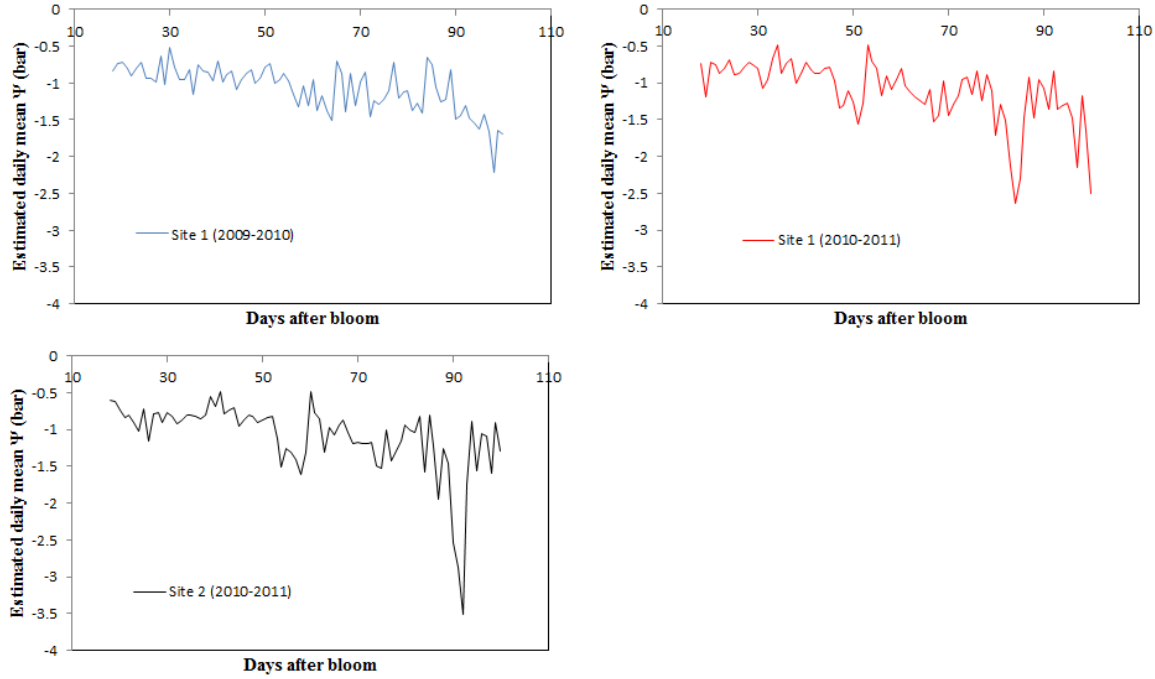


Figure 3 Estimated daily mean stem water potential (ψ) for the seasons and years in which trials were performed. Site 1: 38º29' S 72º 23' W and Site 2: 38º58'S 72º47' W. In brackets, growth season is shown.

APPENDIX 6

Coefficient of dry matter accumulation in 'Brigitta' blueberry fruit

Table 1 Fitted coefficients for curves of dry mass accumulation in fruits[1] growing in plants with high (H), medium (M), medium low (ML) and low (L) fruit load[3]. Site 1: Lautaro, Chile (38º29' S 72º 23' W) and site 2: Freire, Chile (38º58'S 72º47' W).

Treatments	Site	Season	Empirical coefficient[2]				
			A	*B*	*C*	*D*	*E*
H	1	2009-2010	0.1359	5.363	0.2261	4.383 x10^{-3}	3.498 x10^{-5}
M	1		0.2088	4.940	0.2638	6.253 x10^{-3}	5.473 x10^{-5}
ML	1		0.2413	4.606	0.3715	6.585 x10^{-3}	6.181 x10^{-5}
L	1		0.2876	4.159	0.2181	5.280 x10^{-3}	4.834 x10^{-5}
H	1	2010-2011	0.1396	4.278	0.2435	5.923 x10^{-3}	5.130 x10^{-5}
M	1		0.2169	3.604	0.1638	4.046 x10^{-3}	3.979 x10^{-5}
L	1		0.2727	5.245	0. 469	5.148 x10^{-3}	3.964 x10^{-5}
H	2		0.1451	4.866	0.2923	7.332 x10^{-3}	6.483 x10^{-5}
M	2		0.2153	4.766	0.2861	7.075 x10^{-3}	6.240 x10^{-5}
L	2		0.2972	5.492	0.3400	8.119 x10^{-3}	6.910 x10^{-5}

[1]Curve used as model input in Chapter V ($M_d(t) = \frac{A}{1+e^{(b-cDAB+dDAB^2-eDAB^3)}}$), *DAB* is days after bloom.

[2]*P*-values of the coefficients ranged between 0.000173 and 2x10^{-16}.

[3] Fruit load resulting from pruning severity treatments (see Chapter V)

APPENDIX 7

Simulation program

The program is written in R language

```
modemilio<- function(
#Parameters related to Lockart's law (1965)
        Y = 5.38,       # [bar]          Threshold value of turgor pressure for growth
        Y0 = 0,         # [bar]          Minimal turgor in the fruit
        phi = 0.14,     #[ bar^-1 d^-1]  Cell wall extensibility
#Parameters related to the water flux
        Hf = 1,         # [%]                      Relative humidity of the fruit
        acnst = 0.0273, # [dimensionless]          Coefficient for the area of composite membrane
        Lmax = 3.47,    # [g cm^-2 bar^-1 d^-1]    Maximal hydraulic conductivity (g cm-2 bar-1 d-1)
                                                   between stem and fruit
        K3= 0.06        #                          Empirical parameter indicating the rate of change
                                                   of hydraulic conductivity (p1 in Chapter V)
 # Other
        Krat = 0.009,   #                          [g of potassium g^-1 fruit dry mass]
#Osmotic potential given by compounds other than sugars and potassium
        OSMf.autre=1.82         #[bar]
#Conductance of skin
        ro=68.33,               #[cm h^-1]         Fruit surface conductance
# Initial variable
        w0=0.103,                # [g]               Initial water amount in the fresh fruit
#Inputs
        n = 95,                 # number of simulated days

#According to the experimental site, run as:
        temperature = clima11[,"meantemp"],  # [°C]  Daily mean of air temperature
        temperature=clima10[,"meantemp"],
        temperature=climaMDR[,"meantemp"],
        humidite = clima11[,"humedad"],      # [%]   Daily mean of the relative air humidity
        humidite = clima10[,"humedad"],
        humidite = climaMDR[,"humedad"],
        watpot= clima11[,"WatPot"],          #       Vapour pressure deficit
        watpot= clima10[,"WatPot"],
        watpot= climaMDR[,"WatPot"],
#Parameters of dry weight curve
        #Values depend on fruit load treatments
        A=,     b=,     c=,     e=,     f=,
#Initial variable of sugar content
```

```
        sug0=0.2,       # [g (gluc+fruc) g-1dry mass], initial fruit sugar concentration
# Empirical parameters used to calculate the relative rate of transformation of sugars
        k1= 3.15,
        k2= 0.05,
        k4= 0.61,
# Allometric parameters relating fruit area (cm2) to fruit mass (g)
        y=4.24,
        z=0.69,
){
# Parameters
        phi= rep(phimax,n)
        L=Lmax/(1+exp(k3*(1:n)))
# Fruit dry mass growth (g)
        S<-A/(1+exp(b-(c*(1:n))+(e*(1:n)^2)-(f*(1:n)^3)))
#Derivate of fruit growth curve
        derS<--(A*(exp(b -(c*(1:n))+(e*(1:n)^2)-(f*(1:n)^3))*(e*(2*(1:n))
                -c-f*(3*(1:n)^2)))/(1+exp(b-(c*(1:n))+(e*(1:n)^2)-(f*(1:n)^3)))^2)

# Calculation of RGR
        RGR<-(derS)*(1/S)
#Calculation K function
        k=k1*RGR^k4*exp(-k2*(1:n))
# Physiques constants ------------------------------------------------
R<-83                   # [cm3.bar.mol-1.K-1]       Gas constant
SpVw<-18                # [cm3.mol-1]               Specific water volume
Ms.eau <-18             # [g.mol-1]                 Molar mass of water
Ms.glucose<-180         # [g.mol-1]                 Molar mass of glucose and fructose
Ms.potassium<-39.1      # [g.mol-1]                 Molar mass of potassium

###### STRUCTURE OF THE RESULTS
w<-vector (length=n)            # [g]             Water mass of the fruit
tms<-vector (length=n)          # [g DM/gFM]      Dry mass content
Tf<-vector (length=n)           # [cm3.d-1]       Fruit transpiration
fludoentr <- vector(length=n)   # [cm3.d-1]       Water influx
Tfstar<-vector (length=n)       # [cm.d-1]        Transpiration of fruit surface
osmf<- vector(length=n)         # [bar]           Fruit osmotic pressure
turgf<-vector(length=n)         # [bar]           Fruit turgor pressure
osmf.K<- vector (length=n)      # [bar]           Osmotic pressure given by potassium
su<- vector(length=n)           # [g C]           Sugar content

######INITIALISATION
#Masse
```

```
        w[1] <- w0                  # [g]     Masse eau Fruit
#Sugar
        su[1]<-sug0*0.4*S[1]

########
for (i in 1:n)
{

########## INPUT VALUES
#Environment
        Temp<-273.15 + temperature[i]                       # [K]     Temperature
        Ha<- humidite[i]/100                                # [%]     Relative humidity
        Psat<-0.00804817 * exp(0.0546961*(Temp-273.15))     # [bar]   Saturation pressure
#Water potential
        potplant<-watpot[i]
#Fruit traits
        Twght<-w[i]+S[i]                # [g]             Fresh mass of fruit
        Af<-y*(Twght)^z                 # [cm2]           Surface fruit
        ro<-76.71                       # [cm2 h-1]       Fruit surface conductance
#Sugar content
        su[i+1] <-su[i]+(0.41*derS[i])-(k[i]*su[i])
#Concentrations and fruit potentials
        tms[i]  <-S[i] / (w[i]+S[i])                        # [g MS/gMF]    Dry mass content
        Css     <-(su[i]/0.4) / w[i]                        # [g soluble sugars g-1 water]
        Cssm    <- Css / Ms.glucose                         # [mol sugar g-1 water]
        OSMf.suc <- R * Temp * Cssm                         # [bar]   Osmotic potential of sugars
        CK      <-(Krat * S[i]) / w[i]                      # [g potassium g-1 water]
        CKm             <-CK / Ms.potassium                 # [mol potassium g-1 water]
        OSMf.K          <-R * Temp * CKm                    # [bar]   Osmotic potential of potassium
        OSMf    <- OSMf.suc + OSMf.K + OSMf.autre           # [bar]   Fruit osmotic potential
#Calculation of fluxes and potentials
        #Transpiration
                alf     <- Ms.eau / (R*Temp) * Psat         # [adim]
                Tf[i]   <- 24*ro * alf * Af * (Hf - Ha)     # [cm3.j-1]       Transpiration
                Tfstar[i] <- Tf[i]/Af                       # [cm.j-1]        Transpiration of surface
        #Water potentials
        #####Turgor regulation
                numer <- (L[i]*acnst*Af*(potplant+OSMf))-Tf[i]+(Twght*phi[i]*Y)
                denom <- (L[i]*acnst*Af)+(Twght*phi[i])
                Pf <- numer/denom                           #Turgor pressure
                if (Pf<Y) Pf <- (potplant+OSMf) - (Tf[i]/(L[i]*acnst*Af))
                if (Pf<Y0) Pf <- Y0
```

```
        #Calculation water balance in fruit
                fludo <- L[i]*acnst*Af*(potplant - Pf +OSMf)
                delta.eau <- fludo - Tf[i]
                fludoentr[i] <- fludo
 #Integration of state variables
        w[i+1]   <- w[i] + delta.eau
        osmf[i]  <- OSMf        # [bar]        Fruit osmotic pressure
        turgf[i]  <- Pf         # [bar]        Fruit turgor pressure
        }
#Calculation of sugar concentration
        sug=(su[1:n]/0.4)*(1/S)                #gSU/gDM

########## RESULTS
resul <<-
data.frame(Jour=1:n,S=S,w=w[1:n],MF=S+w[1:n],Tf=Tf,Tfstar=Tfstar,fludoentr=fludoentr,tms=tms,
osmf=osmf,turgf=turgf,sug=sug,k=k,su=su[1:n])
resul
}
```