



*Carbon storage
in coconut, oil palm, rubber and mango:
origins, dynamics and consequences for
plantation management*



*Final Meeting of ATP Reserves n° 11/2002,
November 8-9 2006
In CIRAD – Lavalette, Montpellier, France.*

PROCEEDINGS

CONTENTS

| | |
|---|---|
| INTRODUCTION: CONTEXT, AIMS AND PROGRESS REPORT | 5 |
|---|---|

Part 1- Synthesis of Results

| | |
|--|----|
| DYNAMICS OF CARBOHYDRATE RESERVES AS RELATED TO TAPPING IN RUBBER TREE | 11 |
| NATURE, LOCATION AND SEASONAL CHANGES OF NON STRUCTURAL CARBOHYDRATES IN MANGO | 25 |
| THE TRANSITORY CARBON RESERVES IN TWO PALMS: THE COCONUT AND THE OIL PALMS | 39 |
| COCONUT FRUIT QUALITY ASSESSMENT THROUGH MATURATION | 51 |

Development and Use of original analytical and investigations tools

| | |
|--|----|
| RESERVES POOLS IN VEGETATIVE AND FRUIT COMPARTMENTS OF COCONUT AND MANGO TREE: ANALYSIS BY NEAR INFRARED SPECTROSCOPY (NIRS) | 59 |
| BIOCHEMICAL INDICATORS FOR CARBON METABOLISM | 75 |

Part 2- Towards an integrated approach ...

| | |
|--|----|
| ROLE OF CARBON RESERVES IN PALM FAMILY CROPS: AN EMERGING MODEL AND CONSEQUENCES FOR RESEARCH STRATEGY | 83 |
|--|----|

| | |
|---|----|
| PLANT GROWTH MODELS AND ENVIRONMENTAL STRESSES: SHOULD WE BOTHER WITH CARBON? | 89 |
|---|----|

Some examples of plant functioning models

| | |
|---|----|
| MODELING CARBON ALLOCATION AND FRUIT QUALITY IN PEACH TREES | 97 |
|---|----|

| | |
|---|-----|
| ECO PALM: A SIMPLE, PHYSIOLOGICAL MODEL TO SIMULATE MONTHLY VARIATION OF FRUIT PRODUCTION OF AN ADULT OIL PALM PLANTATION | 103 |
|---|-----|

| | |
|---|-----|
| THE « <i>ECOMERISTEM</i> » MODEL: A MODEL OF MORPHOGENESIS AND COMPETITIONS BETWEEN SINKS IN RICE | |
| PART1- | 115 |
| PART2- | 130 |

| | |
|------------------------------------|-----|
| THE N STORES IN TREES: AN OVERVIEW | 143 |
|------------------------------------|-----|

ANNEXES

**“CARBON STORAGE IN COCONUT, OIL PALM, RUBBER AND MANGO:
ORIGINS, DYNAMICS AND CONSEQUENCES FOR PLANTATION MANAGEMENT”**

**INTRODUCTION
CONTEXT, AIMS AND PROGRESS REPORT**

By Isabelle MIALET-SERRA
RU 59, “Integrative Modeling”, CIRAD, AMIS department, Montpellier, France.

Economic Context

The culture of these crops is essential to the populations of the inter-tropical zone which often draw their principal commercial resource. As, it is the case for the majority of the agricultural products, the world rates of these products record full fluctuations which tend to tighten the margins of the producers.

This uncomfortable economic context is worsened by a very irregular production between seasons and between years, even under optimum conditions for culture. *These natural fluctuations remain to be explained.* Lastly, in South East Asia, the appearance of extreme weather events, irregular and presenting increasingly short cycles, are the cause of strong dry periods (related to the phenomenon of *El Niño*), during which the production levels falls drastically in particular for palms.

To face these mixed difficulties, the producers must seek new tools (i) to rationalize their methods of production, (ii) to be able to support their forecasts and their decisions on good analyses of the processes and factors which explain, partly, the development of the production of these crops.

Scientific Context

During the scientific set-up of our ATP project, few research projects were in progress with aims to develop models of functioning and yield forecasting (i) integrating the effects of water constraints sometimes severe and repeated for palms, (ii) in order to propose techniques adapted to orchards management for a best control of the flowering and the fruit quality for mango tree, and (iii) integrating the influence of climatic and soil conditions and the farming practices for rubber tree.

Then, we noted a missing link i.e. *the absence of detailed studies on the carbon storage and its mobilization on these four species.*

In this scientific context, it became interesting for us to develop a generic analysis on which to found the diagnoses and the yield forecasts in an unspecified situation (i) by releasing the importance and the reasons of yield fluctuations under optimum culture conditions and (ii) by determining the processes which lead to the impact of the climatic variations. Our works will fall under this step and will try to bring brief replies.

Challenges were scientific but, also, methodological. Near Infra Red Spectrometry (NIRS) or enzymatic approaches appeared as complementary basic tools for establishing a rapid diagnostic of the trophic status of plants in relation to their yield potentials (quantitative and qualitative).

Challenges were linked to cooperation with our partners. Rational management of farming systems became increasingly a major challenge for them. In this context, the development of yield forecasting tools and tools enabling an evaluation of the climatic effects on production represented major development asset.

Justifications & Aims

Many biologist privilege a trophic approach to explain yield fluctuations. They show, in particular on temperate fruit trees that alternate production can be closely related to the levels of carbon in the plant, directly assimilated by the fronds, but also stored in time in the various vegetative compartments.

Carbon management and allocation remained little documented. But especially the role and the management of an energy supply which carbon reserves can represent are almost ignored. No major work exists on this subject.

To limit our project, we define carbon reserves as soluble and insoluble non structural carbohydrates stored by plant in a temporary and reversible way. For examples, (i) *starch*, is the form of the most important reserve in the plants, revealing so if yes or not, there is a surplus of carbohydrates compared to current demands. For this reason, it is often used like an exclusive indicator of the plant trophic status (ii) *sucrose*, soluble sugar, is the principal sugar of transport and storage.

The carbon reserves play an important role in (i) the *metabolism*: they represent a mobilizable supply of energy; (ii) the *growth*: they can be used as well for the vegetative growth as for the fruit growth as well as assimilates from photosynthesis; (iii) in *defence, resistance* to the stresses, and (iv) *they prevent mortality*.

Storage is a *major function* of the plant as well as the functions most frequently approached which are absorption, transport, growth...

We are based on the *postulate*, very frequently met in the literature, which the woody plants accumulate carbohydrates during excess periods of production and sacrifice them when the utilisation ratio exceeds the production rate.

Palms trees are characterized by continuous and irregular production and long cycles of organ development; Rubber tree by a not reproducer artificial sink entirely managed by human; Mango tree by seasonal waves of growth and fertilization, irregular and sometimes weak peaks of production. These four species have in common long periods of exploitation, many sinks in competition and probably periodic strong demands phases.

Like *basic hypothesis*, we posed that carbon reserves represent a buffer compartment, likely to compensate an insufficient photosynthesis to support the plant demand. We supposed that the reserves status varied during the year, even in optimum conditions, and influenced the production consequently. These crops had an important capacity to constitute reserves. Lastly, we supposed that the *storage determinism* was rather different from determinism usually met in temperate trees with rhythmic growth (imposed mainly by the climate) and supposed whereas the reserves pool variations were less intensive than reserves variations of plants subjected to strong seasonality.

Our *specific questions of research* were:

- *Which are the biochemical nature of the stored reserves, their quantitative importance and their localization?*
- *How do they vary, during seasons, in relation to the vegetative, reproductive growths or the production of latex?*
- *Which role(s) can they play in the functioning of the four studied species?*

The *main aims* were, *on a whole tree scale*

- Analyse reserves *natures*: natures of available soluble and insoluble sugars by enzymatic quantification or HPLC and, on a larger scale, by NIRS;
- Identify "storage" compartments, by locating and sizing them (evaluating reserves quantity) and, so, describe a topological mapping of storage sugars;
- Study reserves variations in time, by considering information previously obtained in relation to environmental constraints (low solar radiation, dry soil and air, etc.) and cultural practices and, so, describe a topological and dynamic mapping of storage sugars;

These objectives were achieved for the four studied species.

- Integrate this information in functioning and yield prediction models, rapidly for the palms, in the longer term, for rubber and mango;

This objective was partly achieved for oil palm tree.

On a finer scale,

- Study the processes involved in the management of carbon reserves. Biochemical indicators responsible for sugar storage and mobilization were required. In addition, for rubber, the process of sucrose filling in laticifer cells was examined taking an immunological approach.

This objective is in progress for rubber tree and for oil palm outside the ATP project.



Part 1- Synthesis of results

DYNAMICS OF CARBOHYDRATE RESERVES AS RELATED TO TAPPING IN RUBBER TREE

By Philippe THALER, Pisamai CHANTUMA, Unakorn SILPI, André LACOINTE, Anne CLEMENT-VIDAL, Poonpipope KASEMPAP, Sornprach THANYSAWANYANGKURA, Eric GOHET, Agnès GUILLIOT.

CIRAD Thaïlande – Montpellier, France; RRIT, Thaïlande ; Kasetsart University, Thaïlande ; INRA – Université Blaise Pascal, Clermont-Ferrand, France.

Introduction

Because of seasonal changes in climatic conditions and developmental variations in source:sink ratio, trees periodically have to sustain growth and other functions when the demand for assimilate temporarily exceeds the current assimilate production. Under such conditions, carbohydrate reserves, defined as resources accumulated in mobilizable form, are to satisfy the demand. Thus, carbohydrate reserves are key factor underlying plantation tree productivity. The chemical nature of these reserves (mainly non structural carbohydrates), and their location and dynamics have been documented for many temperate fruit and forest tree species (Kozłowski 1992; Lacoïnte *et al.*, 1995; Witt and Sauter 1994; Barbaroux and Bréda 2002; Barbaroux *et al.*, 2003). Nevertheless, the mechanisms involved in reserve accumulation and mobilization remain poorly understood. The common concept is that plants store the unused carbohydrates that are produced in excess of current demands for maintenance, growth and reproduction. Reserves are mobilized when current demands exceed assimilate supply (Lacoïnte *et al.*, 1993; Barbaroux *et al.*, 2003). According to this view, reserves are considered a passive buffer. However, this raises the question of how a tree copes with a possible shortage of assimilate, whether in the short term (e.g., in the case of accidental leaf-fall) or in the long term (e.g., in a shaded situation), when adequate reserves cannot be accumulated concurrent with maintenance and growth. A possible explanation is that the concept of reserves as a mere buffer receiving only excess carbohydrates is too simplistic (Cannell and Dewar 1994; Lacoïnte 2000; Le Roux *et al.*, 2001). Furthermore, there is a scarcity of quantitative data on the dynamics of total non-soluble carbohydrate (TNC) concentration in adult trees and particularly in tropical trees at the whole-tree level (Bory and Clair-Maczulajtys 1988, Mialet-Serra *et al.*, 2005).

Rubber exploitation is an unusual system, in that the commercial product, latex, is neither vegetative nor reproductive material but a secondary metabolite comprising mainly poly isoprene. Latex is the cytoplasm of specific cells organized in anastomosed vessels, namely laticifer vessels, within the phloem layer of the trunk bark. In response to tapping (cutting successive thin slices of trunk bark over a period of days), the vessels are severed and latex flows out. Metabolism to regenerate the exported latex is induced and uses assimilates derived from the other sinks (Templeton 1969, Wycherley 1976, Jacob *et al.*, 1985).

Latex biosynthetic rate is commonly modulated by changing tapping frequency or when production is stimulated by the application of ethylene generators. Thus, carbohydrate demand can be artificially modulated, providing a means to study carbohydrate dynamics within the tree (Gohet 1996, Silpi *et al.*, 2006a). Moreover, assessing the time course of accumulation of reserve metabolites at the whole-tree scale will provide insight into the competition between latex production and growth, which may lead to the development of management practices that preserve a balance between these two sinks, a prerequisite for the high and sustainable productivity of rubber plantations (Wycherley 1976, Gohet 1996).

Our experiments were designed for two main objectives: i) to determine whether carbohydrate reserves in trees behave as a buffer or as a competing sink, we assessed how diverting carbohydrate, by tapping adult rubber trees, affected the seasonal dynamics of carbohydrate concentration at different locations along the trunk and, ii) to assess whether knowledge of carbohydrate dynamics in stem wood could help forecast long-term performance of tapping systems, in addition to latex physiological parameters measured by Latex Diagnosis technique ("LD", Jacob *et al.*, 1995).

Material and methods

Experimental site and plant material

The experiments were conducted in a *Hevea brasiliensis* (rubber) monoclonal plots, clone RRIM 600, at the Chachoengsao Rubber Research Center, Eastern Thailand. Mean annual rainfall is 1280 mm year⁻¹ (2001–2002). The dry season lasts about 5 months, from December to April. Tapping generally starts in May and stops at the end of January, allowing 9 months of tapping and a 3-month rest period.

Treatments and sampling

Tapping involves periodically cutting the bark on the trunk, and hence severing latex vessels. It was performed according to the widespread half-spiral system, i.e., with the tapping cut spiralling over half of the trunk circumference. The tapped side is named panel A, and the untapped one is panel B. In a first experiment published in Silpi *et al.*, 2007, control (untapped) trees were compared to tapped trees with or without the use of Ethephon (2-chloroethylphosphonic acid, ET) stimulation. The effect of Ethephon, which stimulates the release of ethylene in the tissues, on latex metabolism is widely documented (d'Auzac *et al.*, 1989), and it is commonly used in rubber plantations to increase latex production per tapping day.

In the present experiment, a new tapping system was tested, namely Double Cut Alternative (DCA), with two half spiral cuts, one on each side (panel) of the tree. Each cut is tapped every four days alternately, so that the tapping frequency at tree scale is $d/2$, whereas it is $d/4$ for each panel. Opening was made at 1.5 m on one side and at 0.8 m on the other side, so as keeping a large distance between tapped areas. In DCA panel A is the low cut and panel B is the high cut. DCA was compared to classical one-cut systems tapped every 2 days ($d/2$) or every 4 days ($d/4$) with 6 stimulations per year. This system was proved to increase latex yield by 25 % as compared to the corresponding $D/2$ system (Gohet and Chantuma 2003). Each tapping treatment included 12 trees (treatment replications).

Cores were sampled on dates based on climate, the annual growth cycle and the latex production cycle: defoliation period (5 February 2003 and 19 January 2004), re-foliation period (6 March 2003 and 20 February 2004), start of tapping or low production period (2 May 2003 and 11 May 2004) and high production period (28 October 2003 and 18 October 2004). Most radial growth occurred between May and October (Silpi *et al.*, 2006b). Samples were taken along the trunk at 50, 100, 150, 200 and 300, 400, 500 and 600 cm above ground. In tapped treatments, samples were taken on both sides of the tree (tapped panel A and untapped panel B), but sampling at 300–600 cm was performed only on the tapped panel. Sampling at 50 and 100 cm was located below the tapping cut, within what is considered to be the main latex regeneration area (Tupy 1973, Silpi *et al.*, 2006a), sampling at 150 cm was within the renewing bark area in tapped panel, and sampling at 200 was above this area. Two samples were taken on the taproot at 10 and 20 cm below the soil surface.

Sampling procedure

At each sampling date, groups of 3 trees from each treatment were sampled. Samples comprised 0.5-cm-diameter, 5-cm long cores, including 1 cm of bark and 4 cm of wood. Samples were collected with a wood auger. Wood and bark were separated. Each core was immediately soaked in liquid N₂ and was kept in a cryo-tube immersed in liquid nitrogen until transfer to the laboratory where it was stored at –80 °C, before freeze-drying at –50 °C. Thereafter, the samples were blended with a ball-blender and stored at –80 °C until analyzed.

Biochemical analysis

Starch, sucrose, glucose and fructose concentrations were analyzed enzymatically. The results were expressed as mg glucose equivalent per gram of structural dry matter ($\text{mg}_G \text{g}^{-1}_{\text{SDM}}$). Soluble sugars are denoted as SS, and total non-structural carbohydrate (starch + soluble sugars) as TNC.

Results

1- Reserves in Trunk Wood

Natures - Starch was the major component in trunk wood, accounting for 79% of TNC in control or untapped treatment (Table 1). SS was almost made of sucrose only. Glucose and fructose accounted for a negligible proportion except at re-foliation, when SS was the highest in re-foliation (February 2003 and January 2004) and starch was the lowest (Figure 4).

Table 1- Main effect of treatment on non-structural carbohydrate concentrations (mg Glu equivalent / g structural DM) in trunk wood, as averaged for all dates and distances from ground (0-2 m.). Newman-Keuls, alpha = 0.05.

| Treatment (df = 3) | Starch | SS | TNC |
|--------------------|---------|---------|---------|
| Control | 51.64 c | 13.99 b | 65.63 c |
| D/2 | 59.40 b | 14.19 b | 73.59 b |
| D/4 | 59.83 b | 15.08 a | 74.92 b |
| DCA | 62.98 a | 14.25 b | 77.23 a |
| F Statistic | 27.40 | 8.03 | 29.16 |
| P | .0001 | .0001 | .0001 |

Effect of tapping - Mean TNC concentration at tree scale was significantly higher in tapped treatments (D/2, D/4 and DCA) than in Control (Table 1). This was a result of higher starch concentrations. Within tapping treatments DCA had the highest starch. But only D4 had a higher SS content than others. Consequently, starch accounted for 81 %, 80% and 82 % of TNC in D/2, D/4 and DCA respectively.

In D/2 and D/4 the untapped side of the tree (panel B) had significantly higher starch and TNC than the tapped one (panel A) (Table 2). However, the latter had still higher content than control. In DCA, there was no difference between the two sides of the tree (panel A and panel B) which were both tapped. They had the same content in starch and TNC than the untapped panel of D/2 and D/4, and therefore higher content than the tapped panel of these classical tapped treatments. The untapped side of D/4 (panel B) had significantly higher SS than the tapped one (panel A).

Table 2- Analysis of variance for combined treatments (tapping x panel) of D/2 and D/4 (Tapped on panel A and untapped on panel B) for all dates and distance from ground (0-2m.). Mean concentration of non-structural carbohydrates (mg Glu equivalent / g structural DM) in trunk wood. Newman-Keuls, alpha = 0.05.

| Treatment (df = 3) | Starch | SS | TNC |
|--------------------|---------|----------|---------|
| D/2 x Panel A | 57.95 b | 14.22 bc | 71.27 b |
| D/2 x Panel B | 61.76 a | 14.15 c | 75.91 a |
| D/4 x Panel A | 57.95 b | 14.78 b | 72.73 b |
| D/4 x Panel B | 61.70 a | 15.39 a | 77.10 a |
| F statistic | 7.78 | 7.91 | 9.54 |
| P | .0001 | .0001 | .0001 |

Vertical pattern in wood (Figures 1 & 2)- In control there was a decreasing bottom-up starch gradient along the trunk. This gradient was steeper in the lower part of the trunk (20-110 cm from ground) than in the upper part (150-300 cm from ground). The overall trend for SS was a slight increasing bottom-up gradient along the axis. Such gradient was opposite to starch, but the range was lower (2.5 mg_G g⁻¹_{SDM} difference between 20 cm to 300 cm from ground). Therefore, vertical patterns in TNC mainly relied on changes in starch along the trunk.

In D/2 and D/4 treatments vertical distribution patterns of starch were much irregular with large variations related to the location of the tapping cut in panel A. Nevertheless, there was an overall significant decreasing bottom-up gradient along the trunk. The vertical gradient was locally disturbed by the presence of the tapping cut at 80-110 cm. distance from ground, with a trend to accumulate starch in wood of previously tapped area, where bark is regenerating. However, vertical pattern in the untapped panel (B) was closer to that of control. Starch content remained higher in panel B of tapped trees than in control all along the trunk.

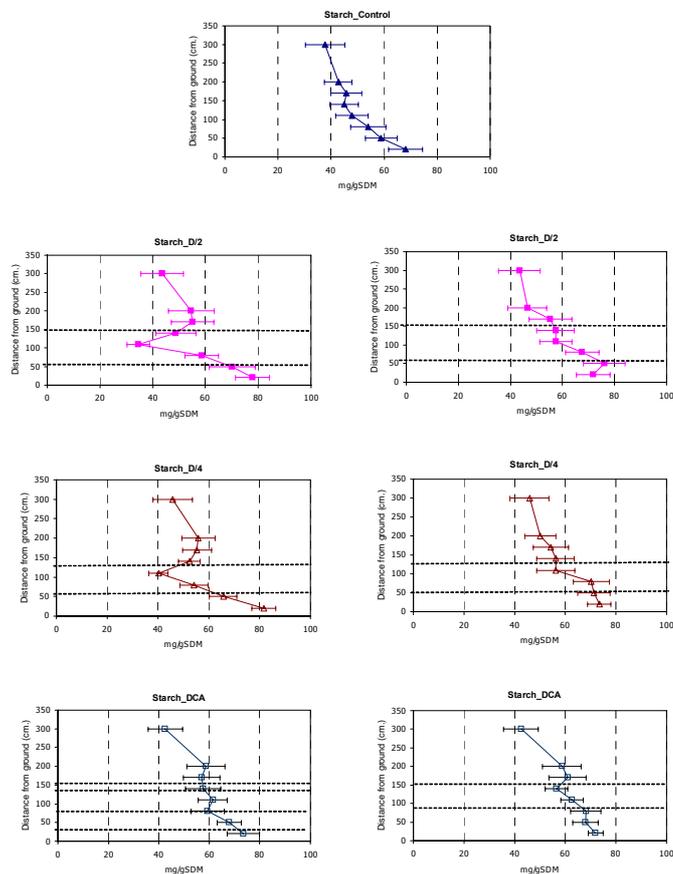


Figure 1- Vertical distribution of starch on panel A (left side) and starch on panel B (right side) in trunk wood. Panel A was tapped in D/2 and D/4 whereas panel B was untapped. Both panel A and B were tapped in DCA. Horizontal dotted lines indicate the location of renewing bark area.

For SS the overall trend was a slight increasing bottom-up gradient along the axis. Such gradient was opposite to starch. It was similar in D/2 than in control, but there was a clear impact of the tapping cut in D/4. SS was the highest at 90 cm in tapped panel and the lowest at the same height in the opposite untapped panel.

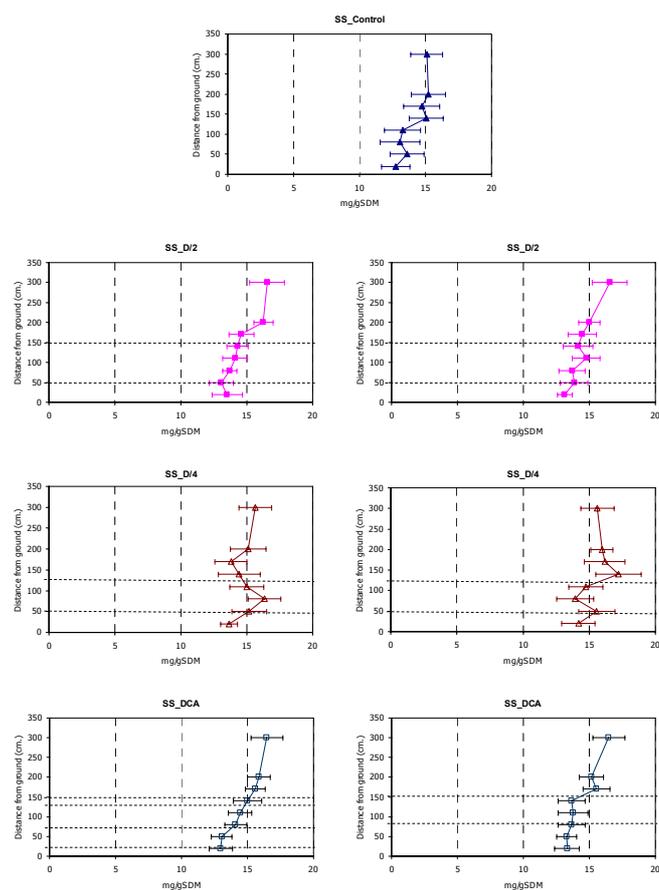


Figure 2- Vertical distribution of SS, total soluble sugar on panel A (left side) and SS on panel B (right side) in trunk wood. Panel A was tapped in D/2 and D/4 whereas panel B was untapped. Both panel A and B were tapped in DCA. Horizontal dotted lines indicate the location of renewing bark area.

In DCA starch and SS were no different between panel A and panel B, both panels being tapped. The overall gradients of starch and SS had the same trend as in control. However, the gradient between 20-200 cm from ground was lower than in control, starch content being much higher all along this part of the trunk for DCA (between 60 to 78 $\text{mg}_G \text{g}^{-1}_{\text{SDM}}$).

Seasonal variation in wood- The overall pattern (Figure 3) showed that, during the first year of observation, the highest TNC concentration was recorded at leaf-fall (February 2003) followed by a huge drop just after complete re-foiation (March 2003) whatever the treatment. A net deposition occurred mainly from May 2003 to leaf-fall (January 2004), i.e. the period, including the rainy season, when both radial growth and (for tapped trees) latex regeneration occurred. SS and starch had opposite trend. In high production to de-foiation stage (February 2003, October 2003 and February 2004, October 2004), starch was high and SS was low, conversely just after leaf-fall, in March, starch was low and SS high. However, variations in TNC were mainly accounted for by variations in starch. During the second year of observation (2004), the drop in starch and TNC content after re-foiation was of lower extent than the previous year. Starch content ranged 43.05-57.38 $\text{mg}_G \text{g}^{-1}_{\text{SDM}}$ in February 2004 whereas it ranged 12.77-32.21 $\text{mg}_G \text{g}^{-1}_{\text{SDM}}$ in March 2003. During the following vegetative season (May 2004 to October 2004) the increase in starch and TNC was not as strong as the previous year.

Along 2 years, mean total TNC concentration in control ranged 31.5-81.2 $\text{mg}_G \text{g}^{-1}_{\text{SDM}}$. At most periods, starch and TNC were higher in tapped treatments (D/2, D/4 and DCA) than in untapped treatment. Average total TNC concentration ($\text{mg}_G \text{g}^{-1}_{\text{SDM}}$) ranged 39.7-93.5, 51.8-96.2 and 42.8-82.4 for D/2, D/4 and DCA respectively.

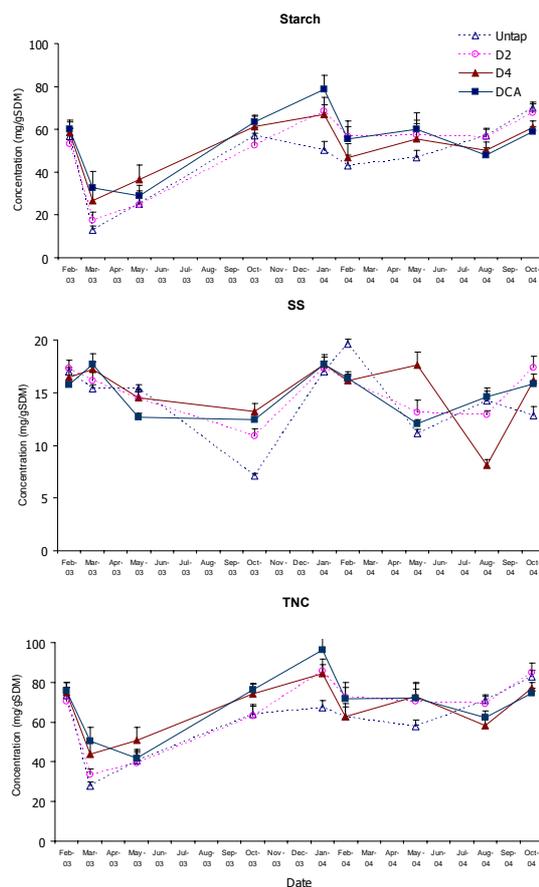


Figure 3- Mean carbohydrate concentration ($\text{mg}_G \text{g}^{-1}_{SDM}$) in trunk wood, up to 600 cm, at 9 sampling dates. February 2003 and January 2004 – leafless stage, March 2003 and February 2004 – at the end of re-foliation, May 2003 and May 2004 – resting period for tapping, October 2003 and October 2004 – high latex production period. starch; SS, total soluble sugars; TNC, total non-structural carbohydrates.

2- Reserves in trunk bark

Natures - SS was the major component of trunk bark, accounting for 73 % of TNC in control treatment (Table 3). SS was almost made of sucrose only. Mean SS was higher in bark ($20.0 \text{ mg}_G \text{g}^{-1}_{SDM}$) than in wood but mean starch content was much lower ($8.4 \text{ mg}_G \text{g}^{-1}_{SDM}$). Consequently, mean TNC was lower in bark ($28.4 \text{ mg}_G \text{g}^{-1}_{SDM}$) than in wood.

Table 3- Main effect of treatment on non-structural carbohydrate concentrations ($\text{mg Glu equivalent} / \text{g structural DM}$) in trunk bark, as averaged for all dates and distances from ground (0-2 m.). Newman-Keuls, $\alpha = 0.05$.

| Treatment (df = 3) | Starch | SS | TNC |
|--------------------|--------|---------|---------|
| Control | 8.34 b | 20.13 a | 28.91 a |
| D/2 | 8.26 b | 20.71 a | 28.97 a |
| D/4 | 8.16 b | 18.66 b | 26.82 b |
| DCA | 8.80 a | 19.32 b | 28.12 a |
| F Statistic | 4.04 | 15.72 | 9.94 |
| P | .0073 | .0001 | .0001 |

SS content was lower in D/4 and DCA than in control and D/2, whereas starch was higher in DCA than in other treatments. Consequently, TNC was lower in D/4 than in other treatments. (Table 3, Table 4).

SS accounted for 72 %, 70 % and 69 % of TNC in D/2, D/4 and DCA respectively. Tapped treatment D/2 and D/4 had significantly higher starch, SS and TNC in panel A (tapped panel) than in panel B (untapped panel). Thus, it was the contrary to what happened in wood. Whereas in DCA, there was no significant difference between the two panels, which were both tapped. Among all panel x treatment combinations, panel A of D/2 had the highest TNC (Table 3, Table 4).

Table 4- 3-way Analysis of variance by combined treatments and panel of Control (untapped tree on panel A), D/2 and D/4 (Tapped on panel A and untapped on panel B) and DCA (both panel A and B were tapped), for all dates and distance from ground (0-2m.). Mean concentration of non-structural carbohydrates (mg Glu equivalent / g structural DM) in trunk bark. Newman-Keuls, alpha = 0.05.

| Treatment (df = 3) | Starch | SS | TNC |
|--------------------|--------|---------|---------|
| D/2 x Panel A | 8.65 a | 21.61 a | 30.26 a |
| D/2 x Panel B | 7.87 b | 19.80 b | 27.67 b |
| D/4 x Panel A | 8.72 a | 19.29 b | 28.02 b |
| D/4 x Panel B | 7.59 b | 18.03 c | 25.62 c |
| F statistic | 10.63 | 25.52 | 27.14 |
| P | .0001 | .0001 | .0001 |

Vertical pattern - Vertical variations in TNC were almost the same as variations in SS. In control treatment SS content did not change along the trunk between 20 to 150 cm from ground. It increased between 150 to 300 cm from ground. (Figure 4). There was a slight decreasing bottom-up gradient in starch along the trunk.

For D/2 and D/4 treatments, vertical distribution patterns of SS were much irregular with large variations related to the location of the tapping cut in panel A. Nevertheless, there was an overall increasing bottom-up gradient along the trunk. SS accumulated at 80-110 cm. distance from ground, where bark is regenerating. In panel B (untapped) trend was the same for both treatments and similar to control. SS in tapped panel of D/4 showed the same trend in both wood and bark. There was no starch gradient in panel B (untapped) for both treatments. In panel A variability was high within D/2 treatment and no clear trend was shown. However, in D/4 the pattern was clearly opposite to SS pattern, starch being depleted between 80 to 110 cm from ground in the bark regenerating area.

In DCA, SS in panel A had the same pattern than in control, although this panel was tapped. In the other side (panel B), which was tapped too, there was a trend to accumulate SS in the bark regeneration area (110-150 cm from ground), although it was not as clear as in treatments with one cut only (D/2 and D/4). Starch tended to be depleted in the bark regenerating area of each panel, but variability within location was high.

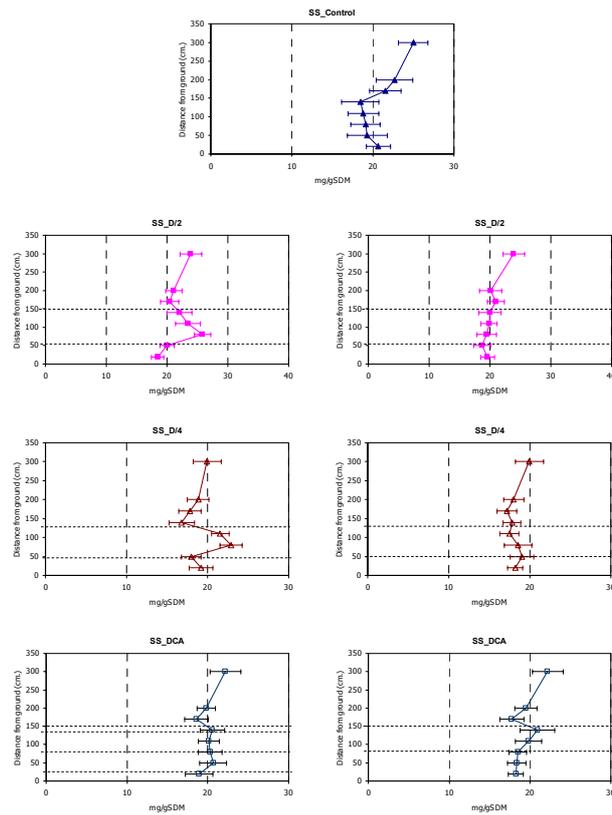


Figure 4- Vertical distribution of SS, TNC on panel A (left side) and SS on panel B (right side) in trunk bark. Horizontal dotted lines indicate the location of renewing bark area.

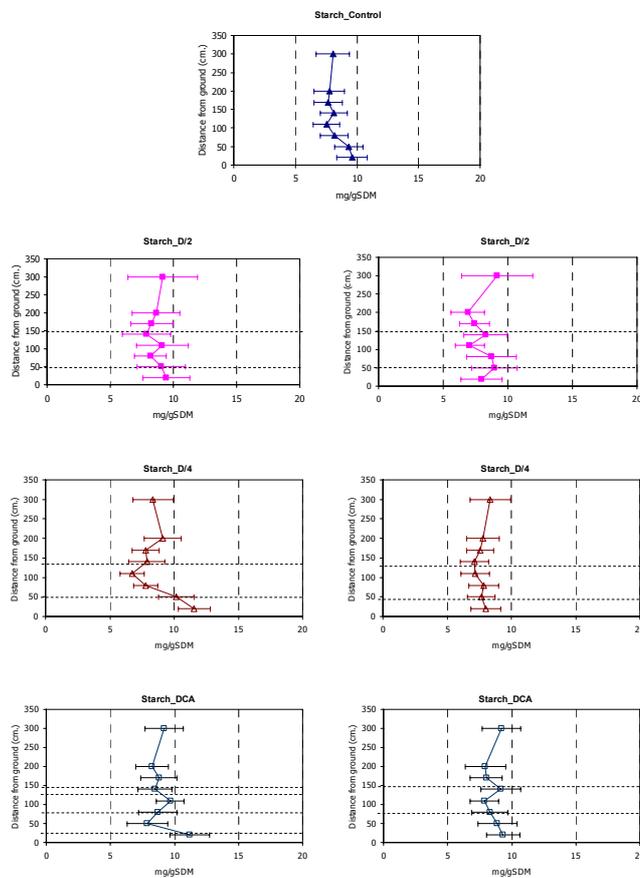


Figure 5 - Vertical distribution of starch on panel A (left side) and SS on panel B (right side) in trunk bark. Horizontal dotted lines indicate the location of renewing bark area.

Seasonal variation in bark - The overall pattern for TNC (Figure 6) was the same as in wood. The highest TNC concentration was recorded at leaf-fall (February 2003) followed by a huge drop just after complete re-foliation (March 2003). A net deposition occurred mainly from May 2003 to next leaf-fall (January 2004). TNC concentration dropped again after complete re-foliation (February 2004) whatever the treatment. From February 2004 to May 2004 (dry season and tapping rest) to high production (October 2004) there was a steady increase. Thus, contrary to results in wood, the seasonal pattern was the same for TNC along the 2 years of observation.

In bark, SS and starch had same trend, except that during the first year most SS deposition occurred earlier (between March and May 2003) than main deposition of starch (between May and October 2003). SS and starch were high in high production stage (October 2003 and 2004) and leaf fall (February 2003 and January 2004), conversely just after leaf-fall, in re-foliation period (March 2003 and February 2004), SS and starch were low (Figure 4). Both changes in SS and starch contributed significantly to variations in TNC along time.

Differences among treatments were not the same along time. However, differences between taped and untapped treatments were not as clear as in wood. Along the year, mean total TNC concentration ranged 26.6-33.0, 27.9-34.8, 24.7-30.7 and 27.2-31.4 $\text{mg}_G \text{g}^{-1}_{SDM}$ for control, D/2, D/4 and DCA respectively.

Starch changed more rapidly in bark than in wood, although to a lower extent.

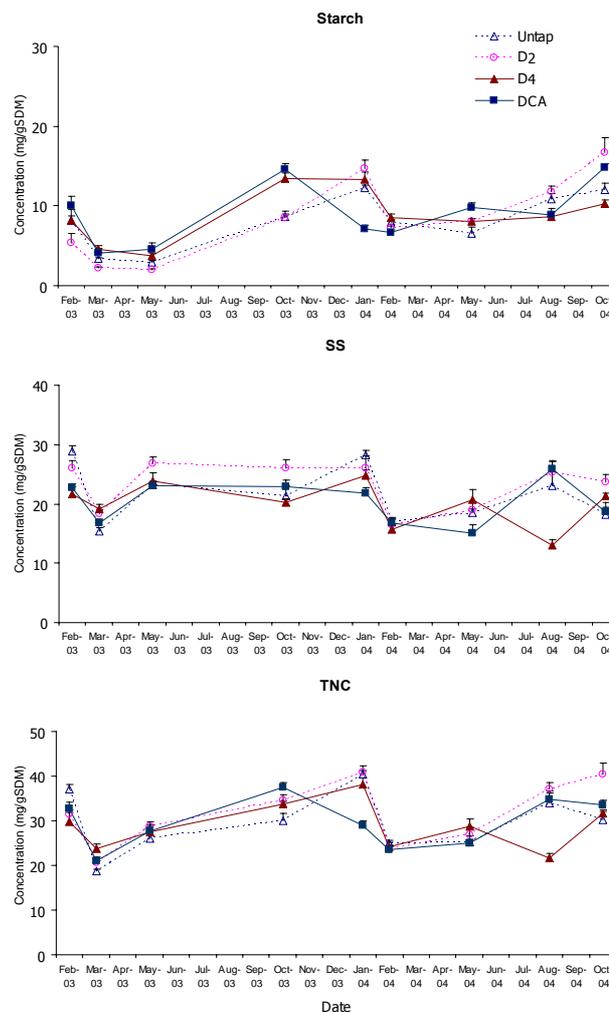


Figure 6- Mean carbohydrate concentration ($\text{mg}_G \text{g}^{-1}_{SDM}$) in trunk bark on panel A, up to 600 cm, at 9 sampling dates. February – leafless stage, March – at the end of re-foliation, May – resting period for tapping, October – high latex production period. St, starch; SS, total soluble sugars; TNC, total non-structural carbohydrates.

3- Reserves in Root (tables 5 & 6)

In inner part of root, mean starch, SS and TNC concentration were no significantly different among treatments (Control, D/2, D/4 and DCA). Starch was the major component of inner part of root, accounting for 76-78% of TNC. In outer part of root, SS was the major component, accounting for 65-77 % of TNC. SS was no significant among treatments, Whereas mean starch and TNC were significantly higher in DCA, D/4 and control than in D/2.

In inner part of root, starch and TNC concentration were significantly higher in lateral root, both samples, than in taproot at 10 cm. and taproot at 30 cm. from ground. In the opposite, SS in tap roots was significantly higher than lateral root. In outer part of root, SS concentration was significantly higher in taproot at 10 cm. and taproot at 30 cm. than in lateral root. But starch in outer part of root was the same as in inner part. Lateral root had higher starch than both tap roots.

The overall pattern starch and TNC of inner part of root and SS and TNC of outer part of root showed the same trend of seasonal variation (Table 13). The highest TNC concentration was recorded at leaf-fall (February 2003) followed by a huge drop just after complete re-foliation (March 2003) whatever the treatment. A net deposition occurred mainly from May 2003 to leaf-fall (January 2004). TNC concentration was steady from re-foliation (February 2004) to high production (October 2004).

Table 5- Mean concentration of non-structural carbohydrates (mg Glu equivalent / g structural DM) in inner part of root and outer part of root, as related to tapping treatment, for all dates and kind of roots homogeneous groups, Newman-Keuls, alpha = 0.05.

| Treatment (df = 3) | Inner part of root | | | Outer part of root | | |
|-----------------------|--------------------|-------|-------|--------------------|-------|---------|
| | Starch | SS | TNC | Starch | SS | TNC |
| Control | 61.11 | 16.50 | 77.61 | 14.29 a | 29.52 | 43.81 a |
| D/2 | 57.19 | 17.82 | 75.00 | 8.53 c | 29.34 | 36.03 b |
| D/4 | 54.19 | 16.93 | 71.13 | 10.90 b | 27.50 | 40.24 a |
| DCA | 55.00 | 15.61 | 70.61 | 14.32 a | 26.32 | 40.64 a |
| F statistic | 2.08 | 2.51 | 2.43 | 18.28 | 1.17 | 6.67 |
| P | .1043 | .0599 | .0663 | .0001 | .3206 | .0003 |

Table 6- Mean concentration of non-structural carbohydrates (mg Glu equivalent / g structural DM) in inner part of root and outer part of root, as related to kind of roots, for all dates and treatments, Newman-Keuls, alpha = 0.05.

| Roots (df = 2) | Inner part of root | | | Outer part of root | | |
|--------------------|--------------------|---------|---------|--------------------|---------|-------|
| | Starch | SS | TNC | Starch | SS | TNC |
| Tap root at 10 cm. | 52.69 b | 17.31 b | 70.00 b | 10.72 b | 29.00 a | 39.72 |
| Tap root at 30 cm. | 50.92 b | 18.74 a | 69.66 b | 11.69 b | 30.45 a | 42.14 |
| Lateral root | 67.00 a | 14.10 c | 81.10 a | 13.36 a | 25.39 b | 38.74 |
| F Statistic | 22.50 | 22.45 | 12.44 | 5.92 | 6.94 | 2.28 |
| P | .0001 | .0001 | .0001 | .0032 | .0012 | .1044 |

Discussion and conclusion

To assess spatial and temporal variations in trunk carbohydrate reserves of rubber trees, we analyzed starch, sucrose, fructose and glucose concentrations in 4-cm-long wood core samples. We observed

consistent patterns in relation to seasonal developmental changes and to the diversion of assimilate in response to tapping.

As in most tree species, starch was the major TNC, and changes in TNC concentration in response to various factors were mainly associated with changes in starch concentration. Mean TNC concentrations in our trunk wood samples were comparable with values reported for beech and oak (Barbaroux *et al.*, 2003), poplar (Witt and Sauter 1994) and walnut (Lacointe *et al.*, 1993). Sucrose was the predominant soluble sugar, and its concentration was more stable than that of starch as a function of location and sampling date.

Seasonal patterns of carbohydrate concentration in control trees were consistent with results reported for deciduous trees (Lacointe *et al.*, 1993, Witt and Sauter 1994, Barbaroux *et al.*, 2003): total carbohydrate concentration decreased sharply after re-foliation, indicating net mobilization, either for direct incorporation in new shoots (including leaf and flowers) or to sustain increased growth respiration (Lacointe *et al.*, 1993). However, unlike temperate species, bud burst in our rubber trees occurred almost without a time lag following leaf fall. Consequently, the leafless period lasted no longer than 2 weeks, and so the requirement for reserves for maintenance was of limited duration. Reserve deposition and radial growth occurred in parallel over the 6-month period when climatic conditions were favourable, similarly to temperate species. Large seasonal variations in starch concentration while sucrose concentration remained stable indicate that sucrose may act as a buffer compartment. When more sucrose from parenchyma was used or transported, more starch was hydrolyzed to compensate for the loss of sucrose.

Effect of tapping- Tapping had a huge impact on carbohydrate concentrations in the trunk and taproot, greatly affecting mean concentration, vertical distribution and seasonal dynamics. The most striking result was that overall starch concentration of tapped trees was higher than that of control trees. Because latex biosynthesis regeneration requires carbohydrate as substrate and as a source of metabolic energy, it is considered that tapping creates an additional sink that diverts carbohydrate from normal functions (Wycherley 1976, Tupy 1985). Previous works (d'Auzac *et al.*, 1989, Jacob *et al.*, 1995, Gohet *et al.*, 1996, 1998) have shown that sucrose concentration within laticiferous vessels drops following tapping and is often a factor limiting latex production. Furthermore, tapping involves repeated wounding of the tree so that the tapped trees not only have to regenerate latex but also undergo sustained stress. Wounding stress is known to increase respiration and thereby carbohydrate use (Uritani and Asahi 1980). Ethylene generation in response to the ET treatment likely increased this effect, because ethylene is known to increase tissue respiration (Abeles 1973). Thus, in contrast to our observations of an increase in starch concentration in response to tapping, we predicted that tapping would induce a depletion of carbohydrate reserves. Moreover, the highest positive difference in TNC concentration between tapped trees and control trees was measured in October, when carbohydrate demand for latex regeneration was highest. Locally, this difference was largest at 50 cm above ground level, within what is considered to be the main latex regeneration area (Tupy 1973, Silpi *et al.*, 2006a). In addition, the tapping system with the highest yield (DCA) was also the one with the highest TNC content. Thus, we conclude that diverting carbohydrate through tapping had a positive effect on carbohydrate, mainly starch, deposition in the trunk parenchyma of the rubber trees. Our results support the view that storage reserves are not necessarily the lowest priority among the competing sinks. When rubber trees were tapped, radial growth was significantly reduced whereas carbohydrate concentration in wood increased. Moreover, the net increase in carbohydrate reserves in response to carbohydrate diversion and stress demonstrated that trees tend to adapt their reserve level to current needs. Priority of allocation to reserves over growth might explain why growth of tapped trees was reduced so much following tapping (Silpi *et al.*, 2006b); Gohet (1996) concluded that the diversion of carbohydrate for rubber biosynthesis is insufficient to explain such a large

decrease in TNC growth. To better understand the partitioning of assimilates among maintenance, growth, latex regeneration and reserves, measurements of total carbohydrates and biomass in all tree parts are needed along with studies on the direct effects of tapping on growth and the metabolism of carbohydrate reserves.

According to the observed dynamics, wood is the long term reserve compartment, which is the more variable, with starch as the reserve component, which has to be hydrolysed into soluble sugar to be used and SS as the available carbohydrate source for transport compartment. On the other hand, bark is the short to medium term compartment, varying less, with starch as relatively stable buffer component and SS as the ready-to-use component.

Vertical patterns of both starch and TNC were more irregular in tapped trees than in control trees, and this irregularity was enhanced by ethylene application. Untapped panels of both d/2 and d/4 trees had higher content than tapped panels and clearly different from those of control trees. The explanation could be that tapping created a sink effect driving carbohydrate into the wood parenchyma of the trunk. In the tapped panel, the use of carbohydrate to regenerate latex reduces the actual accumulation of starch as compared to the untapped panel. Higher starch concentrations were recorded at all heights along the untapped panel compared with the tapped panel, even in its uppermost location during the period of high production, indicating that, although tapping is a local process, the effect on carbohydrate concentration is extensive, affecting both sides of the trunk–taproot axis. This finding has implications for the design and management of the tapping system. Particularly it may explain part of the higher yield in DCA, as each side of the trunk could benefit from the sink effect created by tapping the opposite side. DCA, in which a longer time is allowed for regeneration of latex and where latex is drained alternately from two separated areas on each side of the trunk seemed the more able to accumulate carbohydrate reserves around the latex regeneration area and to mobilize such reserves. Together with the better metabolic profile recorded by latex diagnosis on a trunk scale (Silpi *et al.*, 2006a), this occurrence of high carbohydrate resource in the trunk indicates that performance of DCA is likely to be sustainable.

References

- Abeles, F.B., 1973- Ethylene in Plant Biology. Academic Press, New York.
- Barbaroux C. and Bréda, N., 2002- Contrasting distribution and seasonal dynamics of carbohydrates reserves in stem wood of adult-ring porous sessile oak and diffuse-porous beech trees. *Tree Physiol.*, 22:1201-1210.
- Barbaroux, C., Bréda, N. and Dufrêne, E., 2003- Distribution of above-ground and below-ground carbohydrates reserves in adult trees of two contrasting broad-leaved species (*Quercus Petraea* and *Fagus sylvatica*). *New Phytol.*, 157: 605-615.
- Bory, G. and Clair-Maczulajtys, D., 1988- L'étude des métabolites de réserves. *Bull. Soc. Bot. Fr.* 135 :5-8.
- Cannell, M.G.R and Dewar, R.C., 1994- Carbon allocation in trees: a review of concepts for modelling. *Adv. Ecol. Res.*, 25: 59-104.
- d'Auzac J., Jacob, J-L., Chrestin, H., 1989- Physiology of rubber tree latex, Boca, Raton, Florida, USA: CRC Press. Inc. 470 pp.
- Gohet, E., 1996- The production of latex by *Hevea Brasiliensis*. Relation with growth. Influence of different factors: clonal origin, hormonal stimulation, carbohydrates reserves. Ph D. Thesis, Univ. of Montpellier II, FRA, 343 p.

- Gohet E., Prévot, J-C., Eschbach, J-M., Clément, A., and Jacob, J-L., 1996- Clone, croissance et stimulation, facteurs de la production de latex chez *Hevea brasiliensis*, Plant. Rec. Dev. 3 (1) : 30-38.
- Gohet, E., Dian, K., Prévôt, J-C., Obouayeba, S., Keli, J.Z., d'Auzac, J., Jacob, J-L., 1998- the relationship between latex sugar content, the metabolic activity of the latex bearing system and the production potential of hevea brasiliensis clones. In Symposium on natural rubber (*Hevea brasiliensis*): Vol. II – physiology and exploitation and crop protection and planting methods sessions. Ho Chi Minh City, Vietnam, 14-15 october 1997. Eds International Rubber Research and Development Board (IRRDB).
- Gohet, E. and Chantuma, P., 2003- Reduced tapping frequency and DCA tapping systems: Research towards improvement of Thailand rubber plantations productivity. IRRDB Symposium. IRRDB annual meeting, Chiang Mai, Thailand. 15-16 septembre 2003.
- Jacob, J-L., Prévot, J-C., Lacrotte, R., Eschbach, J-M., 1995- Le diagnostic latex. Plant. Rec. Dev., 2 (2) : 34-37.
- Kozlowski, T.T., 1992- Carbohydrate sources and sinks in woody plants. Bot. Rev. 58 :107-222.
- Lacointe, A., 2000- Carbon allocation among tree organs: a review of basic processes and representing in functional-structural tree models. Ann. For. Sci. 58: 521-533.
- Lacointe, A., Kajii, A., Daudet, F.A., Archer, P., Frossard, J-S., 1993- Mobilization of carbon reserves in young walnut trees. Cambium, Production de bois et développement de l'arbre. Colloque, Société Botanique de France, Paris (FRA), 1992/04/02-03. Acta Bot. Gall. 140 : 435-441.
- Lacointe, A., Sauter, J-J., Ameglio, T., Harms, U., Pellicer, V., Frossard, J.S., 1995- Carbohydrate and protein reserves in trees. In Contribution to forest tree physiology. EUROSILVA Eds, H. Sandermann and M. Bonnet-Masimbert. INRA-Editions « les colloques », n° 76, Paris, pp 273-296.
- Le Roux, X., Lacointe, A., Escobar-Gutierrez, A. and Le Dizès, S., 2001- Carbon-based models of individual tree growth : a critical appraisal. *Ann. For. Sci.*, 58: 469-506.
- Mialet-Serra, I., Clément, A., Sonderegger, N., Roupsard, O., Jourdan, C., Labouisse, J-P., Dingkuhn, M., 2005- Assimilate storage in vegetative organs of coconut (*Cocos nucifera* L.), *Exp. Agr.*, 41 : 141-169.
- Silpi, U., Chantuma, P., Kasempsap, P., Thanisawanyangkura, S., Sangkhasila, K., Gohet, E. and Thaler Ph., 2006a- Is growth performance in rubber (*Hevea brasiliensis*) clones related to xylem hydraulic efficiency? *Can. J. Bot.* 82: 886-891.
- Silpi, U., Chantuma, P., Kasempsap, P., Thaler, Ph., Thanisawanyangkura, S., Lacointe A., Améglio, T. and Gohet, E., 2006b- Sucrose and metabolism distribution patterns in the latex of three *Hevea brasiliensis* clones: Effects of tapping and stimulation on the trunk. *Scale J. Rubb. Res.*, 9 (2) in press.
- Templeton, J.K., 1969- Partition of assimilates. *J. Rub. Res. Inst. Malaya*, 21: 259-273.
- Tupy, J., 1973- Nutrient reserves in roots of fruit trees, in particular carbohydrates and nitrogen. *Plant soil* 71: 401-413.
- Tupy, J., 1985- Some aspects of sucrose transport and utilization in latex producing bark of *Hevea brasiliensis* Müll. *Arg. Biol. Plant.* 27 (1): 51-64.
- Uritani, I. and Asahi, T., 1980- Respiration and related metabolic activity in wounded and infected tissues, In the biochemistry of plants, vol. 2, eds P.K. Stumpf and E.E. Conn. Academic Press, London, pp 463-485.

Witt, W. and Sauter, J-J., 1994- Starch metabolism in poplar wood rays cells during spring mobilization and summer deposition. *Physiol. Plantarum* 92:9-16.

Wycherley, P.R., 1976- Tapping and partition. *J. Rub. Res. Inst. Malaysia*, 24:169-194.



NATURE, LOCATION AND SEASONAL CHANGES OF NON STRUCTURAL CARBOHYDRATES IN MANGO

Frédéric NORMAND, Magalie JANNOYER, Pascal BARANTIN, Gaëlle DAMOUR, Magali DECHAZAL

CIRAD, FLHOR department, Réunion Island & Montpellier, France

Isabelle MIALET-SERRA, Anne CLEMENT, Jean-Luc VERDEIL, Jacques ESCOUTE, Nicole SONDEREGGER, Sandrine LEGROS, Sébastien LAGIER

CIRAD, AMIS, Montpellier, France

Christophe JOURDAN, Fabrice DAVRIEUX

CIRAD-CP, Montpellier, France

Introduction

Two important issues of mango cultivation are i) variable yields which are low on average (world mean: 4 T.ha⁻¹), and ii) alternate bearing which is related to alternate flowering. Flowering appears therefore as a limiting factor for mango production. The identification of factors affecting mango flowering is a prerequisite to build innovative tree management techniques for a better control of flowering and yield. The mango research program of Cirad in Réunion Island is mainly focused on this point. Two complementary approaches are developed:

- the study of tree development and phenology, in particular the reciprocal relationships between vegetative growth and flowering and fruiting,
- the study of acquisition and management of carbohydrates by mango tree, and their relationships with flowering, fruiting and fruit quality. Specific works of the ATP project are integrated in this approach.

Relationships between flowering, fruiting, and carbohydrates availability are documented for several fruit trees, and in particular for mango (Chacko, 1991). For this species, poor flowering and alternate bearing are assumed to be related to low carbohydrates level (Monselise and Goldschmidt, 1982). A common hypothesis is that carbohydrates are necessary in terminal branches for flowering, but they are not sufficient since other factors such as temperature, light, hormones or leaf age may affect floral induction or expression (Davenport and Nuñez-Elisea, 1997). Fruit set, fruit growth and fruit quality are also closely related to carbohydrates availability (Léchaudel, 2004).

Starch is the main molecule for carbohydrates storage. Distribution and utilization of starch reserves within the plant have been studied (Whiley *et al.*, 1989; Davie and Stassen, 1997; Stassen and Janse van Vuuren, 1997; Davie *et al.*, 2000) and the annual cycle of starch has been described in mango: starch accumulates in various woody and non-woody organs from harvest to flowering, and then declines during fruit growth. Fruit is the main sink for starch, but flowering and expanding roots or shoots are also important sinks. However, these studies are limited as they are generally related to a part of the tree, such as leaves or shoots (Pathak and Pandey, 1976; Veera and Rao, 1977; Whiley *et al.*, 1989), or they consider large scale components of the whole tree: roots, bark, wood, shoots, leaves and fruit (Davie *et al.*, 2000). Studies at a fine scale on starch and soluble sugars in the whole tree are lacking. It is however of interest for a better understanding of carbohydrates cycling in the tree.

Our hypotheses for the studies carried on during the ATP project were that carbohydrates balance of a mango tree is driven by flowering and fruiting, which are major sinks, and by cultural practices such

as irrigation that affect sources or sinks strength. On the other hand, we assume that carbohydrates availability affects flowering and fruiting.

The objectives of these studies were i) to provide a detailed map of carbohydrates location within a mango tree, ii) to study changes of this carbohydrates map in relation to the phenological cycle and irrigation, and iii) to investigate the relationships between carbohydrates and flowering and fruiting. Other works have been carried on during the ATP project, such as the histological study of vegetative mango tree organs and the characterisation of root activity. They are not presented in this paper which is focused on the carbohydrates map in mango tree.

Materials and methods

The experiment was conducted in Reunion Island (21°06' S, 55°32' E) during the 2002-2003 phenological cycle on a 13 year-old mango trees orchard, cv. Cogshall, grafted onto 'Maison Rouge'. Tree spacing was 6m x 4m. Water was supplied to half of the trees on a 100% evapotranspiration basis three times a week from fruit set in September 2002 until beginning of rainfall in January 2003. The other trees were not irrigated.

Sampling calendar

Mango phenological cycle on the experimental orchard (Figure 1) was characterised by flowering from the end of August to the end of September, generally in two consecutive flushes. Harvest spread from beginning of December to beginning of February. Vegetative growth started weakly at the end of fruit growth and occurred mainly during the hot and rainy season from January to April. June and July corresponded to a rest period for the tree, during which it is assumed that carbohydrates accumulate before floral induction (Chacko, 1991). Six sampling dates were determined on the basis of this cycle and of the expected carbohydrates levels in trees (Figure 1).

At each sampling date, 3 trees were sampled in the irrigated part of the orchard. Sampled organs of one of these trees were then separated, oven-dried and weighted. The root system was excavated according to the Voronoi technique and different types of roots were separated and treated as previously for aerial organs. Biomass distributions among the organs within a mango tree were calculated from these results. At the end of harvest and during the 2003 rest period, 3 additional trees per date were also sampled in the non-irrigated part of the orchard in order to evaluate the effect of irrigation on carbohydrates content (Figure 1).

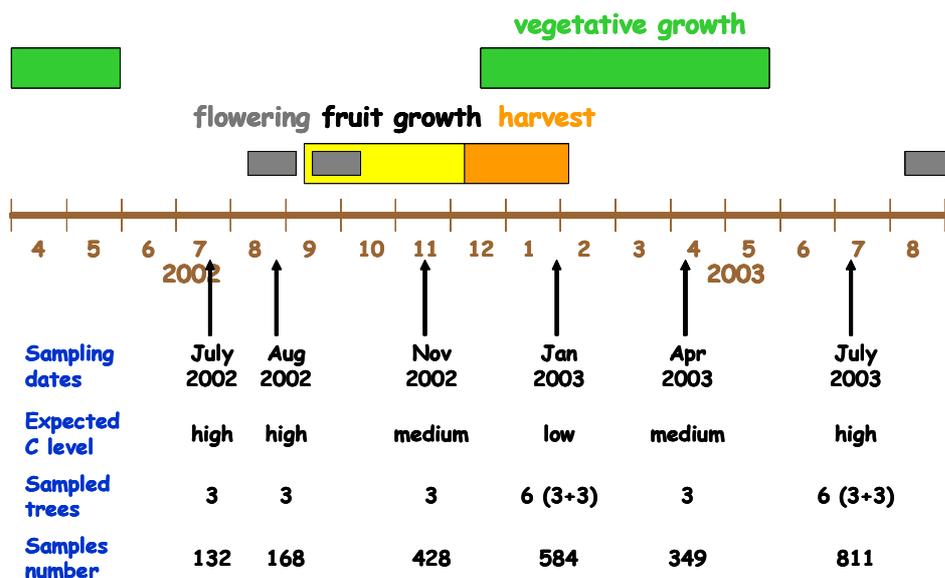


Figure 1- Phenological cycle of mango trees on the experimental orchard and sampling calendar based on expected tree carbohydrates levels.

Sampling scheme

Twenty-six vegetative organs were sampled (Table 1). They were mainly chosen according to their relative age in order to put forward a sugar concentration gradient within the tree woody framework or between leaves of different ages. By definition, scaffold branch is stemmed from the trunk, and first level scaffold branch is the first level of branching of a scaffold branch. In the same way, second and third level scaffold branches are the first level of branching of respectively a first and a second level scaffold branch. Eighteen of these organs were present for all sampling dates and corresponded to the organs of the trees at the first sampling date. The other eight organs corresponded to growth units and leaves that appeared during the 2003 vegetative growth period. Seven reproductive organs were sampled (Table 1). Four of them were related to different stages of panicles development; and three were related to fruit (peel, pulp and seed).

The sampling scheme took into account the hypothesis of reciprocal relationships between carbohydrates and flowering and fruiting. First, we choose first scaffold branches as base units to sample within the tree canopy, and there were as many replicates of organs located above first scaffold branch as first scaffold branches in a tree. This feature arose from the fact that scaffold branches seem to have independent behaviour for flowering and fruiting in mango and it was interesting to verify if this might be related to contrasted carbohydrates status of scaffold branches. Second, nature of terminal growth units (GU) during the 2002 fruiting period was taken into account from the 'fruit growth' sampling date. This nature was 'vegetative' when terminal GU did not flower, 'flowering' when it flowered but did not bear fruit, and 'fruiting' when it bore fruit. Nature of a given terminal GU was assigned to the sub-terminal GU that bore it and to the GUs appeared during the 2003 vegetative growth period. This feature arose from the fact that panicles and fruits are sinks that might affect local carbohydrates balance. This effect was investigated on the sub-terminal and terminal GU that bore panicle and/or fruits, and also on the new GU produced during the 2003 vegetative growth period.

Samples drawing and treatment

Radial core samples were drawn on large woody organs (diameter > 3 cm) with a Pressler drill. Organ pieces were drawn for smaller branches and roots, and for fruit peel and pulp. Complete organs were drawn for growth units, leaves, panicles and seeds. Samples were rapidly brought to laboratory after drawing. They were weighted, frozen in liquid nitrogen and then stored at -40°C. They were freeze-dried (-55°C, 0.02mbar, 65h) and weighted again in order to determine dry matter content (Damour and Normand, 2006). They were then crushed in a ball mill and sent for sugar analyses to the RU 59 laboratory, CIRAD-Montpellier.

Flowering intensity, number of fruits and individual fruit weights were recorded on each scaffold branch of studied trees during the 2002 and the 2003 fruiting periods.

Table 1- Mango tree organs sampled during the study. The presence denotes if the organ was present at all phenological stages ('always'), or not (temporarily).

| Compartment | Organ | Presence |
|------------------------|--|-------------|
| Roots | Fine roots ($\emptyset < 2\text{mm}$) | Always |
| | Medium roots ($2 < \emptyset < 10\text{mm}$) | Always |
| | Coarse roots, 2 m from stump | Always |
| | Coarse roots, 0.1 m from stump | Always |
| | Taproot | Always |
| | Stump | Always |
| Aerial woody framework | Rootstock stem | Always |
| | Grafting point | Always |
| | Cultivar stem | Always |
| | Scaffold branch | Always |
| | First level scaffold branch | Always |
| | Second level scaffold branch | Always |
| | Third level scaffold branch | Always |
| Growth units | Sub-terminal growth unit | Always |
| | Terminal growth unit | Always |
| | New growth units (4 types) | Temporarily |
| Leaves | Third level scaffold branch leaves | Always |
| | Sub-terminal growth unit leaves | Always |
| | Terminal growth unit leaves | Always |
| | New growth unit leaves (4 types) | Temporarily |
| Panicles | Floral bud bursting | Temporarily |
| | Spreading inflorescence | Temporarily |
| | Inflorescence in full bloom | Temporarily |
| | Dry inflorescence | Temporarily |
| Fruits | Peel | Temporarily |
| | Pulp | Temporarily |
| | Seed | Temporarily |

Results

Data analysis is still in progress and main available results are presented here.

1- Biomass distribution within a mango tree

The 13 year-old mango tree excavated during the 2002 rest period had a biomass of 178.4 kg. Vegetative biomass was rather stable from 2002 rest period to the end of harvest and then increased, in relation to new vegetative growth and likely to secondary growth of woody organs (Figure 2). The tree excavated at the time of fruit growth was slightly smaller than the others. Biomass distribution among main vegetative compartments (roots, wood, and leaves) was stable between phenological stages as well. Roots represented 29.9% of vegetative biomass. Contributions of wood and leaves to vegetative biomass were respectively 56.3% and 13.8%. At the time of flowering and at the end of harvest, panicles and fruit biomass were respectively 3.4% and 11.0% of overall tree biomass (Figure 2). Fruits production was high during the studied phenological cycle (about 90 kg per tree). It fell significantly to 51 kg per tree the following year, illustrating the phenomena of alternate bearing.

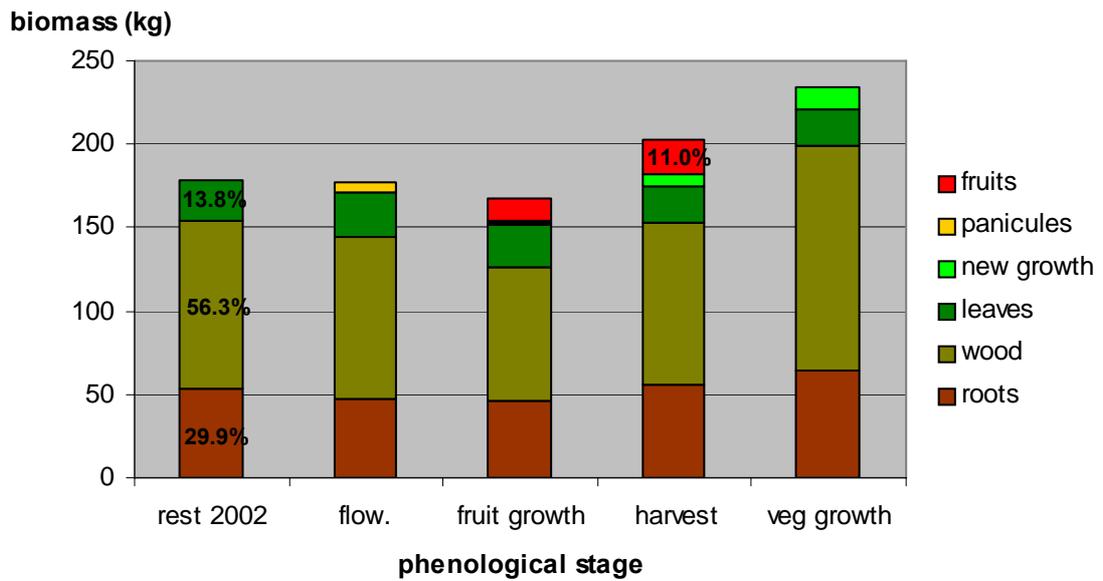


Figure 2- Biomass distribution among main compartments of a mango tree at five phenological stages. Contributions of each vegetative compartment during 2002 rest period and of fruits at harvest are indicated within bars.

Vegetative biomass distributions among the different organs of the root system on one hand (Figure 3), and of scaffold branches on the other hand (Figure 4) were rather stable along the phenological cycle, suggesting a strong hierarchy among these organs. More than 60% of roots biomass was located in coarse roots (32.8%) and in taproot (28.9%). Fine roots only represented 6.0% of roots biomass. Third level scaffold branch was the main organ in scaffold branches, representing 50.0 to 66.4% of scaffold branches biomass (Figure 4). Sub-terminal and terminal GUs accounted for 7.0% of scaffold branches biomass at the time of 2002 rest period.

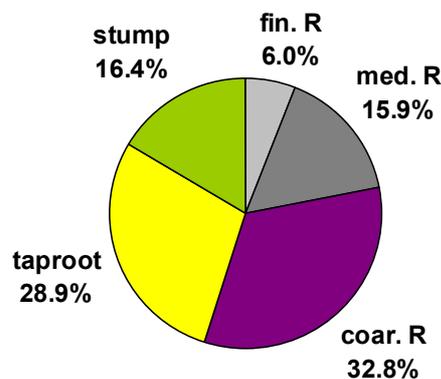


Figure 3- Biomass distribution among different types of roots of mango root system at the time of fruit growth (total root system biomass: 46.4 kg). fin. R: fine roots; med. R: medium roots; coar. R: coarse roots.

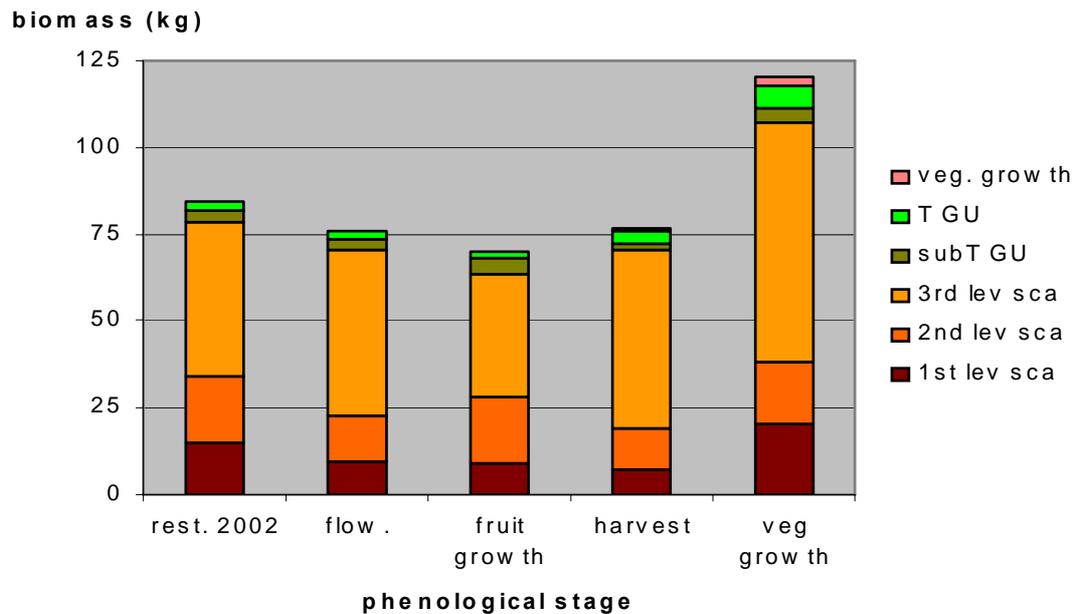


Figure 4- Biomass distribution among different organs of mango scaffold branches at five phenological stages. 1st lev sca: first level scaffold branch; 2nd lev sca: second level scaffold branch; 3rd lev sca: third level scaffold branch; subT GU: sub-terminal growth units; T GU: terminal growth units; veg growth: new growth units appeared during vegetative growth period.

2- Nature, biomass and location of non-structural carbohydrates in mango tree

Starch was by far the most important carbohydrate in mango tree. At the time of 2002 rest period, starch biomass was 84.2% of total non-structural carbohydrates (NSC) biomass (Σ NSC=32.9 kg). *Sucrose* represented 13.5% of this biomass. *Glucose* and *fructose* were minor carbohydrates (respectively 1.1 and 1.3% of total NSC biomass).

Although the order of importance of these NSC did not change at the time of harvest, their relative contribution to the total NSC biomass was affected by fruits and their high concentration in sucrose and fructose. Total NSC biomass of the tree, including fruits, was then 38.5 kg and sucrose represented 34.7% of this biomass. Glucose and fructose contributions were respectively 2.0 and 5.2% of NSC biomass. We will now focus on the results on the major carbohydrates in mango tree, *sucrose* and *starch*.

Starch biomass decreased from 2002 rest period (27.7 kg) to the end of harvest (22.4 kg). It was mainly located in roots and wood (Figure 5). Growing fruits were rich in starch (19.9% of total starch biomass), mainly located in the pulp. But at harvest, only 6.3% of starch biomass was located in fruits, mainly in seed. On the opposite (Figure 6), *sucrose* biomass increased sharply between 2002 rest period (4.4 kg) and the end of harvest (13.4 kg). *Sucrose* biomass was distributed similarly among wood, leaves and roots (respectively about 45, 31 and 24%) at the time of 2002 rest period and at the end of harvest. At the end of harvest, more than half of total *sucrose* biomass of the tree (58.5%) was located within fruits and was therefore exported by harvest.

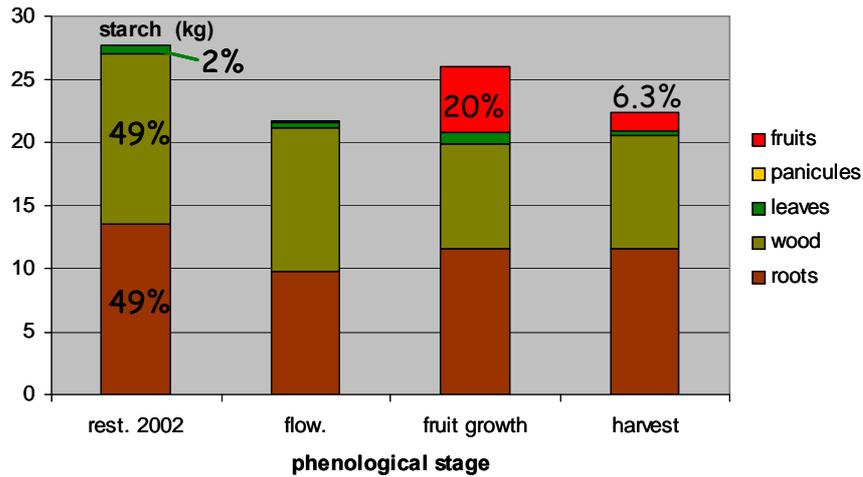


Figure 5- Starch biomass distribution among main compartments of a mango tree from 2002 rest period to the end of harvest. Contributions of each vegetative compartment during 2002 rest period and of fruits during fruits growth and at harvest are indicated on the graph.

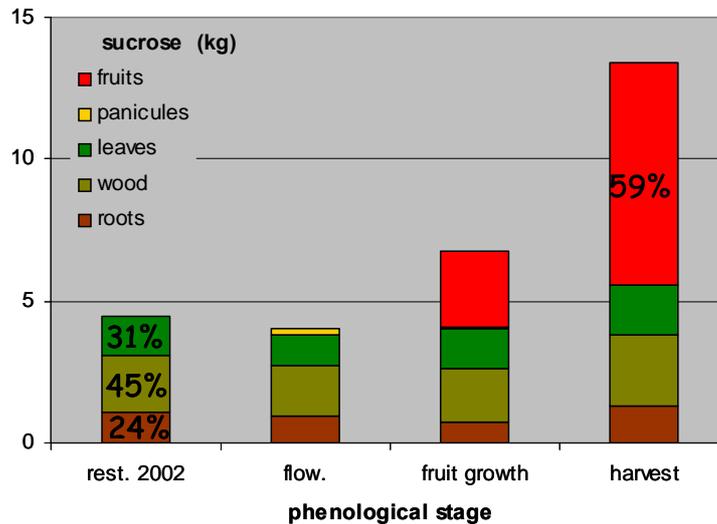


Figure 6- Sucrose biomass distribution among main compartments of a mango tree from 2002 rest period to the end of harvest. Contributions of each vegetative compartment during 2002 rest period and of fruits at harvest are indicated on the graph.

When NSC biomass were pooled (Figure 7), NSC biomass decreased in vegetative compartments from 2002 rest period to the end of harvest whereas total NSC biomass of the tree increased in relation with fruit growth. This pattern suggests that fruits NSC come in part from NSC located in vegetative compartments and in another part from photosynthesis enhanced by fruit growth (Urban *et al.*, 2003). Panicules were not important sinks for NSC as they contained 2.4% of total NSC biomass. On the opposite, fruits represented strong sinks. As early as fruit growth period, they contained about 30% of total NSC biomass. This NSC amount was exported at harvest, leaving a depleted tree in comparison with its NSC status during the rest period.

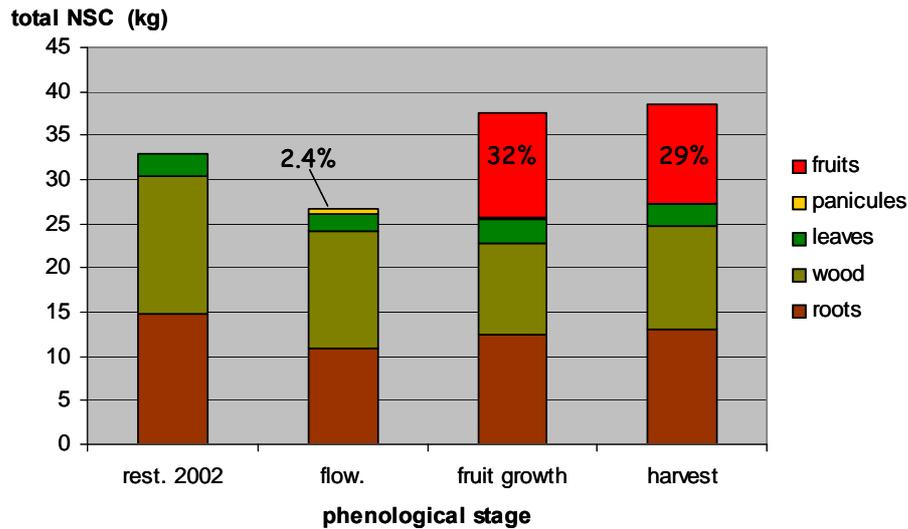


Figure 7- Non structural carbohydrates (glucose + fructose + sucrose + starch) biomass distribution among main compartments of a mango tree from 2002 rest period to the end of harvest. Contributions of panicles during flowering and of fruits during fruits growth and at harvest are indicated on the graph.

3- Sucrose and starch concentrations in vegetative mango tree organs, and their changes during phenological cycle

Pattern of variation of sucrose and starch concentration among organs is described at the time of flowering. Their changes with phenological stages are then presented.

Sucrose concentration varied significantly from 11 to 41 mg.g⁻¹MS between organs of the mango woody framework, from fine roots to terminal growth units (Figure 8A). It was quite stable from fine roots to third level scaffold branches, with a minimum in taproot, and increased in sub-terminal and terminal GUs where it reached a maximum. Sucrose concentration also varied significantly, from 34 to 47 mg.g⁻¹MS, in leaves of different ages (Figure 8B). The younger the leaves, the higher the sucrose concentration. This pattern of sucrose concentration in woody organs and leaves is likely related to light exposure and photosynthesis as leaves of terminal GUs are at the canopy periphery and have therefore a better access to light.

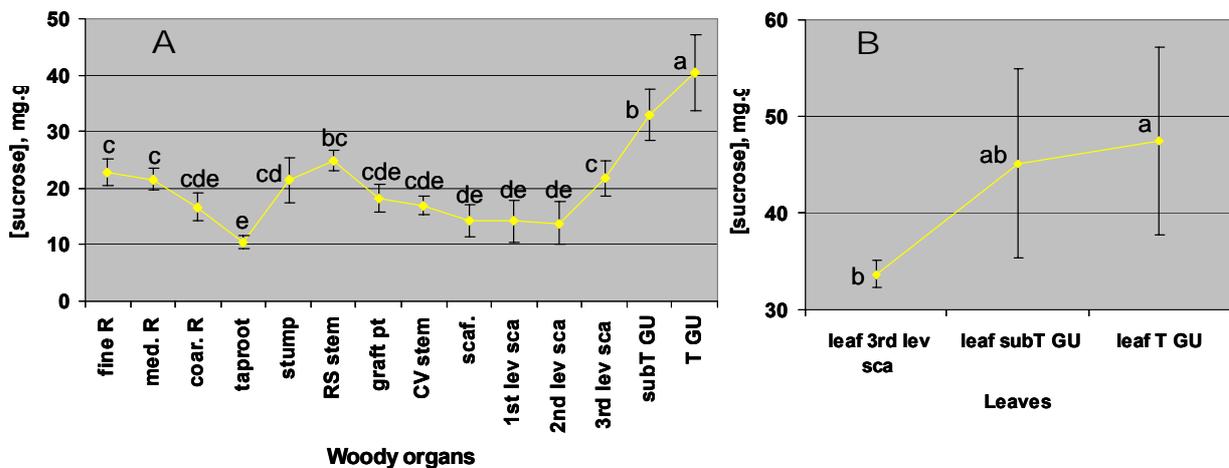


Figure 8- Pattern of sucrose concentration (mean ± standard deviation) of organs of mango tree woody framework (A) and of leaves of different ages (B) at the time of flowering. Means with different letters are significantly different (Tukey's test, P<0.05). fin. R: fine roots; med. R: medium roots; coar. R: coarse roots; RS stem: rootstock stem; graft pt: grafting point; CV stem: cultivar stem; scaf.: scaffold branch; 1st lev sca: first level scaffold branch; 2nd lev sca: second level scaffold branch; 3rd lev sca: third level scaffold branch; subT GU: sub-terminal growth units; T GU: terminal growth units.

Starch concentration was high in old woody organs, in particular in old root (Figure 9A). Taproot, stump and coarse roots had the highest starch concentration (250 to 300 mg.g⁻¹MS) and appeared therefore as storage compartments. Starch concentration was quite stable in old aerial woody organs (rootstock stem to second level scaffold branches), with mean values between 133 and 175 mg.g⁻¹MS. Finally, the younger the organ, for roots and aerial woody framework, the lower starch concentration (Figure 9A). These results are consistent with starch storage in xylem parenchyma (Normand *et al.*, 2006). Storage capacity is higher in old wood in relation to a large presence of these parenchyma. However, starch appears to accumulate mainly in the old root system as compared to the aerial woody framework. These results on starch concentration are also consistent with results presented here on starch biomass. Root biomass represented 29.9% of total tree biomass (Figure 2), but 49.0% of total starch biomass of the tree (Figure 6). Starch concentrations were much lower in leaves than in woody organs and tended to increase in younger leaves (Figure 9B). Although differences were not significant at the time of flowering, they were significant for other phenological stages.

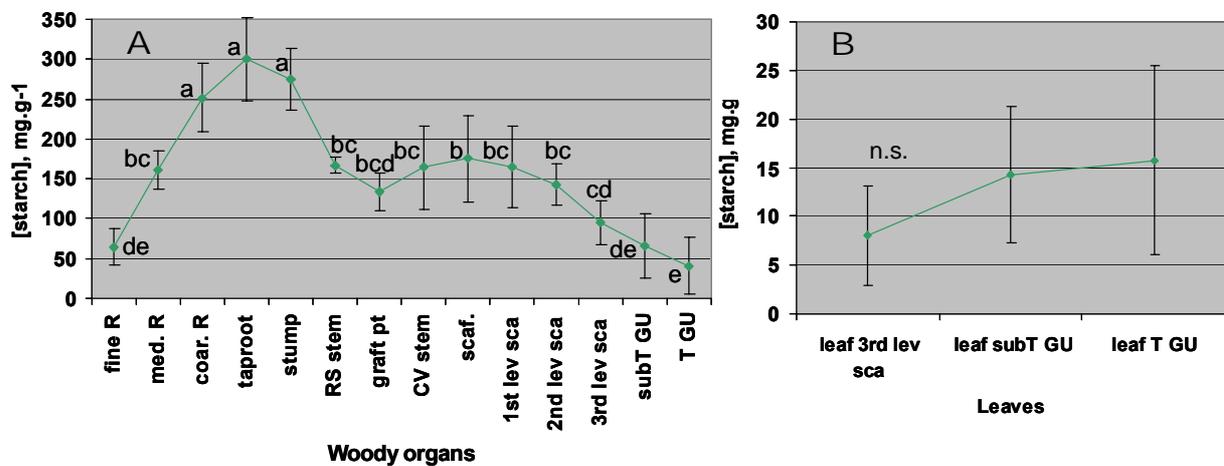


Figure 9- Pattern of starch concentration (mean \pm standard deviation) of organs of mango tree woody framework (A) and of leaves of different ages (B) at the time of flowering. Means with different letters are significantly different (Tukey's test, $P < 0.05$). Organ abbreviations are as in figure 8.

For most organs, sucrose concentration varied significantly with phenological stage (Figure 10), in particular for fine, medium and coarse roots and for organs of scaffold branches. After harvest, sucrose concentration in sub-terminal, terminal and new GUs were significantly lower than before harvest. During the 2003 rest period, sucrose concentration in GUs at sub-terminal (labelled T2 GU) and terminal (labelled T1 GU) position were significantly lower than in GUs in similar position (respectively subT GU and T GU) during the 2002 rest period (Figure 10).

Same kinds of results were obtained for starch concentration in tree organs that were affected by phenological stages (Figure 11). A strong effect of phenological stage on starch concentration was observed in fine, medium and coarse roots for the root system, and in third level scaffold branches, sub-terminal and terminal GUs. These results suggest that fruits growth mobilizes starch at a local scale (third level scaffold branches to terminal GUs) and farther in the root system. However, more detailed data analyses are necessary to reinforce this hypothesis. As for sucrose, starch concentration was significantly lower during the 2003 rest period than during the 2002 rest period in GUs at sub-terminal and terminal position.

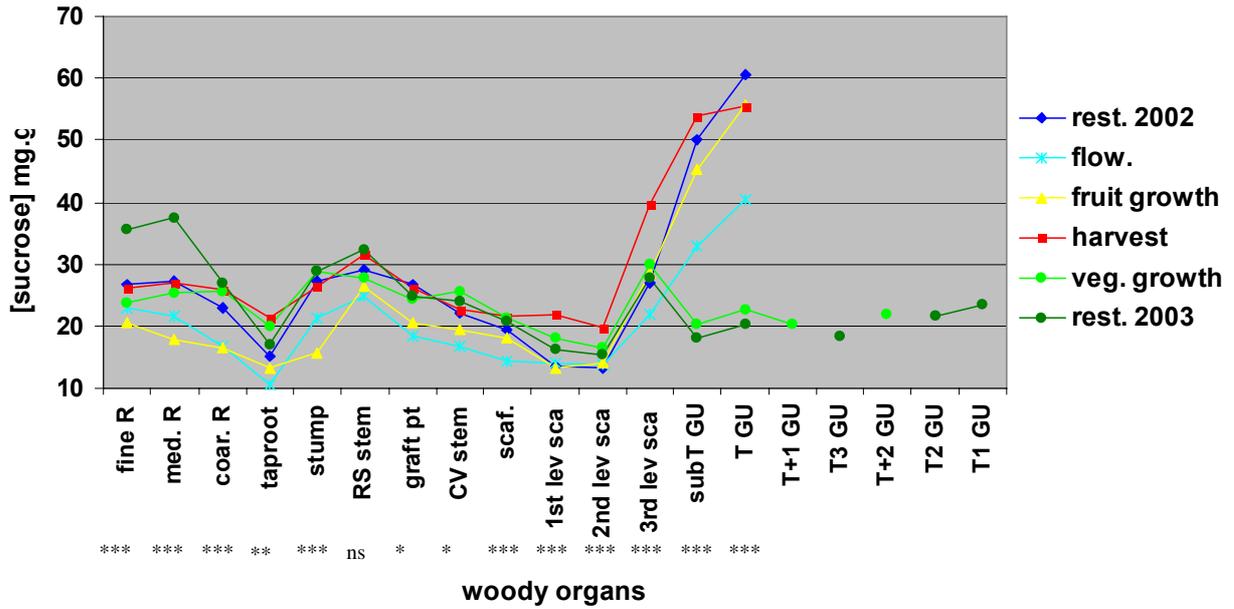


Figure 10: Effect of phenological stage on sucrose concentration of organs of mango tree woody framework. It is indicated for each organ if the effect of phenological stage on sucrose concentration is significant or not. ns : non significant; * : P<0.05; ** : P<0.01; *** : P<0.001. Organ abbreviations are as in figure 8.

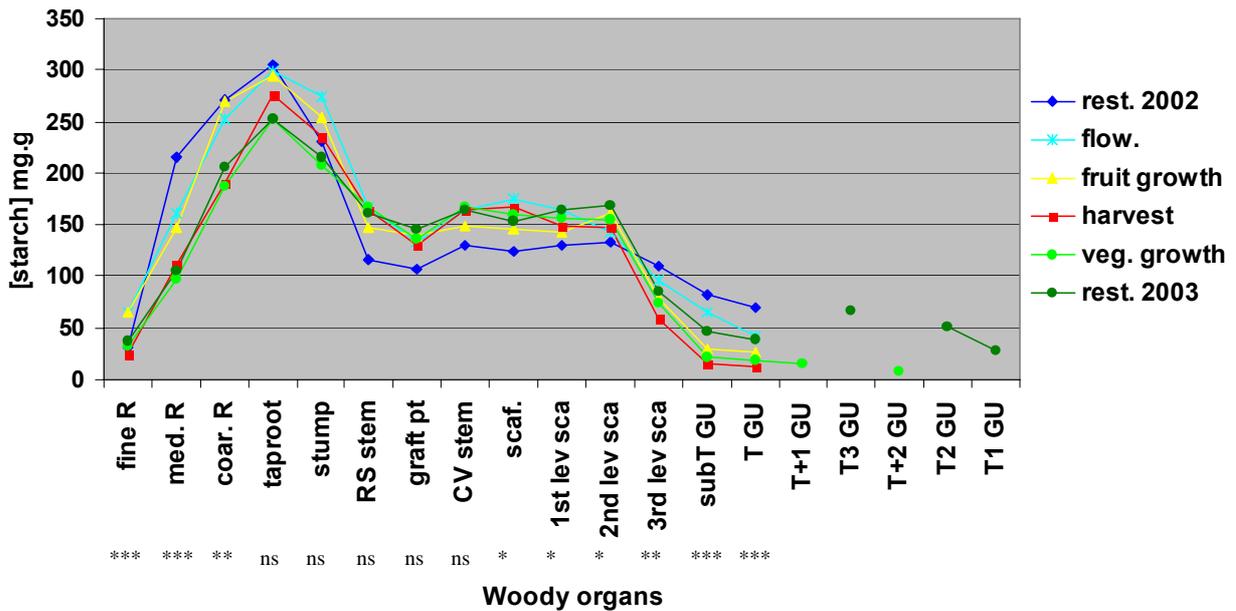


Figure 11: Effect of phenological stage on starch concentration of organs of mango tree woody framework. It is indicated for each organ if the effect of phenological stage on starch concentration is significant or not. ns : non significant; * : P<0.05; ** : P<0.01; *** : P<0.001. Organ abbreviations are as in figure 8.

Discussion

Our dataset on NSC in mango is original and detailed at a fine scale. As specified at the beginning of the Results section, data analysis is still in progress. We have presented here partial and descriptive results. It is not possible at that time to answer to the different objectives of the study, nor to have a complete view of non-structural carbohydrates management within a mango tree. However, these results lead to some conclusions and hypothesis.

This study provides a detailed map of NSC location within a mango tree and describes their changes during phenological cycle. NSC can be characterised in terms of concentration or in terms of amount located in each organ. The large number of sampled organs allows to study NSC changes at a fine scale, or to consider large compartment such as roots, wood and leaves.

NSC concentrations are affected by phenological stages. These changes seem to be mainly related to fruits production which is major sinks for carbohydrates in mango. During the 2002 heavy crop, fruits represented 11% of total tree biomass, but 30% of total NSC biomass of the tree, mainly sucrose, and to a lesser extend fructose and starch. Fruits growth seems to mobilize starch at a local scale (terminal and sub-terminal GUs, until third level scaffold branches), and at a distant scale in the root system. Starch concentration in old woody framework between these locations does not seem to be affected by fruits growth. Panicles biomass is not negligible (3.4% of total tree biomass during flowering), but they are not a major sink for NSC (2.4% of total NSC biomass of the tree). However, starch begins to be mobilised in sub-terminal and terminal GUs during flowering.

On the basis of NSC concentration in organs, NSC reload, and particularly starch reload, is not complete during the vegetative season following a heavy fruits production. Under the hypothesis of a relationship between NSC status of the tree and flowering, this result suggests a role for NSC in alternate bearing in mango. This question of NSC reload should however be also analysed in terms of NSC biomass.

In conclusion, NSC changes, and particularly starch changes, during the phenological cycle are mainly driven by fruits production in mango. A high fruits production leads to a depletion in NSC amount within the tree (organs are differently affected), which is not refilled during the following vegetative season. NSC amounts and/or concentrations are then lower during the next rest period, in particular in terminal GUs that are supposed to flower. Flowering intensity and fruits production were lower the next year. But it does not, at this stage, demonstrate the relationship between NSC and flowering.

Prospects

Several prospects are proposed at this stage. They concern the continuation of data analysis according to the objectives of the study. The results presented here also suggest new experiments to validate some of them.

The first prospect is to continue data analysis:

- To study tree and NSC biomass after harvest in order to have a complete view of these variables during the whole phenological cycle.
- To study the reciprocal relationships between NSC biomass and/or concentration and flowering intensity and yield: does NSC biomass and/or concentration in scaffold branches (organ(s) to be determined) during rest period affect the subsequent flowering intensity and fruits production? And on the opposite, does fruits production at the tree or scaffold level affect NSC biomass and/or concentration at and after harvest in these entities?
- To study at a local scale the effect of the nature of terminal GU on NSC biomass and/or concentration during phenological cycle.
- To study the effect of irrigation on NSC changes

Some results and hypotheses presented in this paper, such as the major role of fruits production on NSC changes in mango, might be validated and deepened with appropriate experiments. These new experiments will benefit from the methodological experiences developed during the present study: improved sampling and drying procedures, choice of pertinent organs for NSC analysis, rapid and cheap determination of NSC concentration using NIRS technology (subject to accurate equations of calibration for most sugars and organs in mango).

Finally, these results could be synthesised in a model of NSC changes within a mango tree which could be coupled with existing models: a biochemical photosynthesis model at the leaf level (Urban *et al.*, 2003) and a fruit growth model (Léchaudel *et al.*, 2007). The objective is a model of flowering (dates and intensity) and of production for mango (harvest dates, yield, fruit quality) (Jannoyer *et al.*, 2006).

References

- Chacko, E.K. 1991. Mango flowering – still an enigma. *Acta Horticulturae*, 291:12-21.
- Davenport T.L. and Nuñez-Elisea R. 1997. Reproductive Physiology. In: *The Mango, Botany, Production and Uses*, edited by Litz R.E., CAB International 69-146.
- Davie, S.J. and Stassen, P.J.C. 1997. Mango model: starch distribution in different tissues of "Sensation" mango trees of varying ages. *Acta Horticulturae* 455:143-150.
- Davie, S.J., Stassen, P.J.C. and Grove H.G. 2000. Starch reserves in the mango tree. *Acta Horticulturae* 509:335-346.
- Damour G. and Normand F. 2006. Distribution and variability of dry matter contents in mango tree cv. Cogshall. Communication presented at *8th International Symposium on Mango*, 5-10/02/2006, Sun City, South Africa. To be published in *Acta Horticulturae*.
- Jannoyer M., Urban L., Léchaudel M., Normand F., Lauri P.E., Jaffuel S., Lu P., Joas J., Ducamp M-N. 2006. An integrated approach for mango production and quality management. Communication presented at *8th International Symposium on Mango*, 5-10/02/2006, Sun City, South Africa. To be published in *Acta Horticulturae*.
- Léchaudel M. 2004. Croissance et qualité organoleptique du fruit chez le manguier (*Mangifera indica*) : analyse expérimentale et modélisation de l'effet de la disponibilité carbonée et hydrique. Thèse de doctorat de l'Institut National Agronomique Paris-Grignon, Paris, France. 146 pages
- Léchaudel M., Vercambre G., Lescourret F., Normand F. and Génard M. 2007. An analysis of elastic and plastic fruit growth of mango in response to various assimilate supplies. *Tree Physiology*, 27, 219-230.
- Monselise, S.P. and Goldschmidt, E.E. 1982. Alternate bearing in fruit trees. *Horticultural Reviews* 4: 128-173.
- Normand F., Lagier S., Escoutes J., Verdeil J-L. and Mialet-Serra I. 2006. Starch localisation in mango tree: histological observations. Communication presented at *8th International Symposium on Mango*, 5-10/02/2006, Sun City, South Africa. To be published in *Acta Horticulturae*.
- Pathak, R.A. and Pandey, R.M. 1976. Effects of age and position on the accumulation of dry matter, sugars and acid hydrolysable polysaccharides in mango (*Mangifera indica* L.) leaves cv. Dashehari. *Progressive Horticulture* 8(1):41-50.

- Stassen, P.J.C. and Janse van Vuuren, B.P.H. 1997. Storage, redistribution and utilization of starch in young bearing "Sensation" mango trees. *Acta Horticulturae* 455:151-159.
- Urban L., Le Roux X., Sinoquet H., Jaffuel S. and Jannoyer M. 2003. A biochemical model of photosynthesis for mango leaves: evidence for an effect of the local source/sink balance on photosynthetic capacity. *Tree Physiology*, 23, 289-300.
- Veera, S. and Rao, V.N.M. 1977. Studies on certain endogenous constituents of shoots in relation to flowering in mango. II. Changes in dry matter, total carbohydrates, total nitrogen and C:N ratio. *Orissa Journal of Horticulture* 5(1):24-34.
- Whiley, A.W., Saranah, J.B., Rasmussen, T.S., Winston, E.C. and Wolstenholme, B.N. 1989. Effect of temperature on growth, dry matter production and starch accumulation in ten mango (*Mangifera indica* L.) cultivars. *Journal of Horticultural Science* 64:753-765.



THE TRANSITORY CARBON RESERVES IN TWO PALMS: THE COCONUT AND THE OIL PALMS

By Isabelle MIALET-SERRA, Jean-Pierre CALIMAN, Anne CLEMENT-VIDAL, Olivier ROUPSARD, Nicole SONDEREGGER, Sandrine LEGROS, Fahri SIREGAR, Christian NOEL, Alexia PRADES, Christophe JOURDAN, Lilik WIDIASTUTI, Jean-Pierre LABOUISSSE, Murielle CROISETU, Thomas FOUGEROLLES, Kamal KANSOU, Samuel SAYAG, Jacques ESCOUTE, Jean-Luc VERDEIL, Fabrice DAVRIEUX, Aurélie DEVEAU & Michael DINGKUHN.

CIRAD, Montpellier, France ; SMARTRI, Libo, Riau estate, Sumatra, Indonesia ; VARTC, Saraoutou, Espiritu Santo, Vanuatu.

Introduction

The culture of these two palms is essential to the populations of the inter-tropical zone which often draw their principal commercial resource (i.e. copra or copra or palm oils), and a multitude of by-products for local use for the coconut. As it is the case for the majority of the agricultural products, the world rates of copra (in particular) and, to a lesser extent of the palm oil, record full fluctuations which tend to tighten the margins of the producers. This uncomfortable economic context is worsened by a very irregular production between seasons and between years, even under optimum conditions for culture. These natural fluctuations remain to be explained. Lastly, in South East Asia, great zone of coconut and oil palm cultures, the appearance of extreme weather events, irregular and presenting increasingly short cycles, are the cause of strong dry periods (related to the phenomenon of El Niño), during which the production levels falls drastically. A bad effect of haze, a consequence of forests fires during dry periods, appears too, in particular in oil palm culture area.

To face these mixed difficulties, the producers must develop new tools to rationalize their methods of production and to be able to support their forecasts and their decisions on good analyses of the processes and the factors which explain, partly, the development of the production of these two palms. These tools will help them for forecasting, for decisions to manage the plantations (logistic, inputs...) and for supporting agronomic diagnostics.

Carbon storage could, partly, explain the production development of these two palms. Like basic hypothesis, we posed that carbon reserves represent a buffer compartment, likely to compensate an insufficient photosynthesis to support the plant demand. We supposed that the reserves status varies during the year, same in optimum conditions, and influences the production consequently. The massive morphology of these two palms and their anatomy (Croisetu, 2003; Fougerolles, 2004) let think that they have an important capacity to constitute reserves. Lastly, in such systems functioning continuously we supposed that storage determinism is rather different from that usually met in species with rhythmic growth (imposed mainly by the climate). We supposed whereas the reserves pool variations will be less marked than those of plants subjected to strong seasonality.

The objectives of this study were to (i) characterize the chemical nature, localization and quantity of such vegetative storage in coconut and oil palm, (ii) characterize the annual and seasonal dynamics of the various reserve pool and, (iii) model their probable role as a physiological buffer as source and sink activities fluctuate. The hypothetical buffer role of assimilate reserves will be tested by the experimental removal of sinks (fruits) and sources (fronds) and by the application of a water stress by root ablation.

Materials and Methods

Palms Characteristics

Coconut and Oil palms, which are closer to the grasses, are characterized by a simple organization (figure 1A): a single stem, fronds whose physiological age is known and who are arranged according to a well ordered geometry. The growths of the vegetative and fruit compartments are concomitant. So in optimum conditions for water, their growths appear continuous. Under such conditions, the growth of the vegetative compartments is stable; the growth of the fruit compartment appears rather irregular. We supposed that there are more or less strong competitions between vegetative and fruit compartments, but also between fruit compartment, which will need energy demands with variable intensity according to their various stages of maturity. Simultaneous presence of many sinks at various maturity stages makes these competitions difficult to dissociate (figure 1B). Development of fruit compartment is very long (from bunch initiation to harvest, 2 years for oil palm and 3 years for coconut). Severe conditions, at a given moment, condition the levels of production several months later. *For all these reasons, these simple systems appear complex and make delicate their studies.*

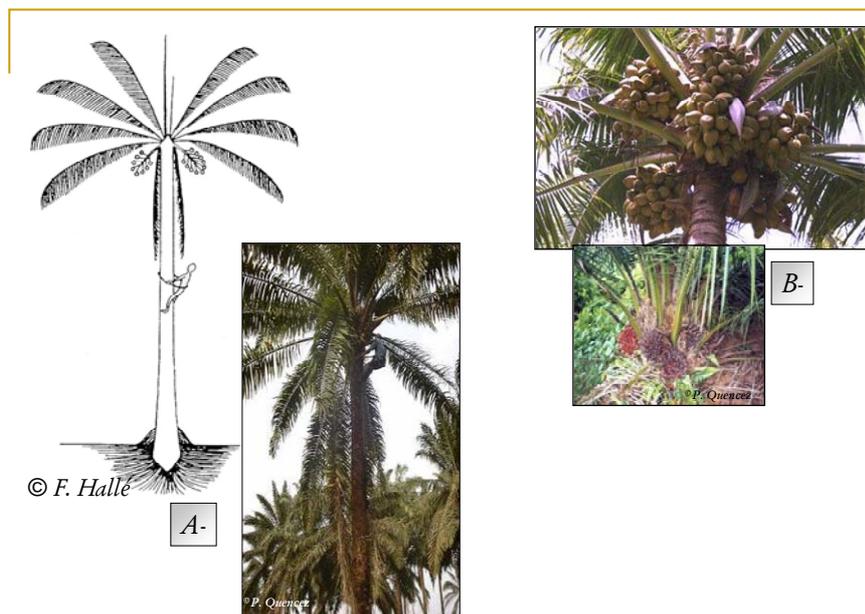


Figure 1- (A) An adult palm; (B) Bunches at different stages of maturation in crown of coconut and oil palm

Experimental sites and plant materials

On coconut, the experiment was conducted on the coconut plantation of the Vanuatu Agricultural Research and Training Centre (VARTC) in Saraoutou (Espiritu Santo, Vanuatu, Southern Pacific) and, on the oil palm experimental plantation of SMARTRI (the Research Institute of Pt. Smart Tbk.) in Kandista Estate (Riau Province, Sumatra, Indonesia). On these two sites, the climate is tropical oceanic and favourable for the growth of these two palms. Planting materials studied for coconut was a hybrid between the Vanuatu Red Dwarf (VRD) and the improved Vanuatu Tall (VTT); for oil palm a tenera hybrid from Dami Dura and Dami Pisifera. Coconut palms were 17 years old and Oil palms 9 years old at the beginning of our study. Population densities were, respectively, 160 plants ha⁻¹ for coconut and 143 plants ha⁻¹ for oil palms.

Protocols

To describe the nature of carbon reserves and their quantitative distribution within the plant, we set up a first trial (trial 1) with four felling campaigns during 18 months. It consisted in sampling all the vegetative compartments (stem, crown and root system) of 28 palms. At the beginning of our studies, we did not have any hypothesis to locate reserves in these two palms. This was the reason why we have chosen, initially, a broad and systematic sampling. To follow and describe the seasonal variation of the reserves, with the vegetative and fruits growths we set up a second trial (trial 2) on 16 palms. It consisted in sampling every two months the principal compartments of storage (the stem and petioles of fronds), in relation to the biometric measurement of vegetative and fruit growths.

Applied Treatments

Equilibrium between sources and sinks had been modified artificially. Four treatments were applied on trial 2: *Control*, *bunch ablation*, *severe leaf pruning* and *root ablation*. Each treatment comprised four palms (as replication), followed during 18 months for coconut and during 35 months for oil palms every 15 days for growth measurements (every 2 months for reserves sampling).

- "*Bunch ablation treatment*", which served to limit fruit demand and remove a major part of the demand for assimilates, consisted of removing all inflorescences just before fertilization. 100% inflorescence ablation was realized over our total observation period.
- "*Severe leaf pruning treatment*", which served to limit, in theory, assimilate supply, consisted of removing on a monthly basis all leaves except the 14 (coconut) or 17 (oil palm) youngest on each crown. Defoliation of leaf from 30 to 14 fronds per coconut reduced light interception of 30% and from 40 to 17 fronds per oil palm of 22% per oil palm.
- Water deficit is probably the most important climatic factor affecting coconut and oil palm yield. "*Root ablation treatment*", which served to limit water and minerals supply, consisted of removing, *a priori*, 50% of root system with 2 opposite circle pits (100 cm depth and 40 cm wide) dug to 20 cm of stem base.

Results & Discussions

1- Natures, Localization and Quantitative Importance of sugars

Coconut - Sucrose was the main form of storage in an adult coconut (81% of Total Sugars (TS)). It represented approximately more of the four fifths of the total pool, whatever the plant compartment or the season of culture. It was present, in particular, in the stem. *Starch* seemed to be a minor sugar. It was mainly localised in the sub-apical zone of the stem, forming in this point, a pocket whose volume can be very variable between replications. This variation remained a point badly encircled in our study. *Monosaccharides (glucose + fructose)* represented energy directly usable in all the biological processes, more than a reserve form. They were, always, present at low levels than sucrose, in particular in the stem, where they represented in general less than 15% of TS. In two cases, we could observe an accumulation of these sugars: (i) at the beginning of trial 1, in pathological stress situation (ii) on the treatment of severe leaf pruning. Each time, we were in conditions of reduced carbon assimilation.

At the scale of the whole plant, the total sugars quantity represented 8% of the total dry biomass (30 kg per coconut or 4.8 t per ha). Its quantitative importance was large and sufficient to sustain full growth rates during 50 days in the absence of fresh assimilates.

Oil Palm – Glucose was the main pool of sugars in an adult oil palm tree (53% of TS) in all vegetative compartments followed by *sucrose* and *starch* (20% each of TS) and little *fructose* (only 7% of TS). They were present, in particular, in the stem. Starch did not seem to be a minor sugar, but it was mainly localised in the sub-apical zone of the stem like for a coconut palm.

At the scale of the whole plant, the total sugars quantity represented 20% of the total dry biomass (127 kg per palm or 18.2 t per ha). So its quantitative importance was very large and sufficient to sustain full growth rates during 7 months in the absence of fresh assimilate and under our culture conditions.

Applied treatments effects – On the stem, *monosaccharides* pool level was higher on "severe leaf pruning treatment", in reduced carbon assimilation conditions for coconut; *sucrose* and *starch* pools higher and *monosaccharides* pool lower on "bunch ablation treatment" for oil palm, however the total was significant higher on this treatment.

On petioles, *TS* pool level was higher on "bunch ablation" and "root ablation" for coconut; lower for "severe leaf pruning" and "root ablation" for oil palm, in this case "bunch ablation" was not significant different than control.

The effects of applied treatments differed according to species. These observations should enable us to better define thereafter the role of each sugar in the functioning of these two species.

2- Variations in time & effects of applied treatments

Vegetative and Fruit growths

The *vegetative growths* on these two palms recorded little fluctuations in time. Consequently carbon allocation to vegetative compartments was stable. No treatments effect was observed for coconut; significant effects for oil palm. In this case, fronds (i.e. emission rate of new fronds) and stem (i.e. height increment) growths were more intense on "bunch ablation", less intense on "root ablation" (Siregar, 2006). *Plasticity of vegetative compartments (e.g. fronds and stem) was higher on oil palm than on coconut.*

The *yield* components of these two species were (i) the bunches number, in particular female bunches number for oil palm, (ii) the fruit number per harvested bunch & (iii) the fruit size (or biomass), and consequently the bunch size.

- (i) The number of bunches depended on abortion rate for the both species and on sex ratio¹ for oil palm. Abortion rate was equal to zero for coconut in our culture conditions; equal to 13% on control for oil palm. No seasonal and inter annual variations were observed. But treatments effects were observed on abortion rate for oil palm: higher on "severe leaf pruning" and on sex ratio, lower on "root ablation" and on "severe leaf pruning";
- (ii) For *coconut*, during our experience, the fertilized flowers number and fruit loading increased significantly 14 months after the beginning of our observations. This event was exceptional and very variable between coconut trees. The number of harvested nuts per bunch was higher during wet season (in March). Fertilized flowers number, fruit loading and pruning rate of immature nuts after fertilization were higher on control. For *oil palm*, the number of fruits per harvested bunch was higher during wet season. The number of fertile fruits per bunch was higher on control treatment, the number of unfertile fruits (or parthenocarpic) lower.
- (iii) For *coconut*, the harvested nuts biomass was stable between seasons and applied treatments. For *oil palm*, fruit biomass varied between seasons (lower during wet season) but not with applied treatments.

Consequently, for the both species, fruit compartment was more plastic than vegetative compartments. For coconut, a strong regulation of production was observed in term of nuts number, not in term of produced biomass; seasonal variations were observed (with higher yield in wet season). For oil palm, the biomass of a harvested bunch was unchanged whatever the seasons but more bunches were harvested in wet season. So yield was higher in wet seasons. Yield intensities decreased with the applied treatments (i.e. « Root Ablation » & « Severe Leaf Pruning ») on oil palm only.

¹ Sex-ratio = female bunches / [female bunches + male bunches + aborted bunches]

Reserves pools

No effects of seasons and applied treatments were observed on natures and localization of reserves pools.

Coconut – Three phases were isolated over our trials:

- *Phase*①: The initial experimental conditions (coconut recovery following a pathological stress) enabled us, during the first year, to observe the reserves recovery (figure 2), during a convalescence period affecting more photosynthesis than fruit growth. The starch, little represented, varies then very clearly, and could constitute a good indicator of a temporary surplus in carbohydrates compared to the demands. After hydrolysis, this sugar would then contribute punctually to reach again an optimal productivity.

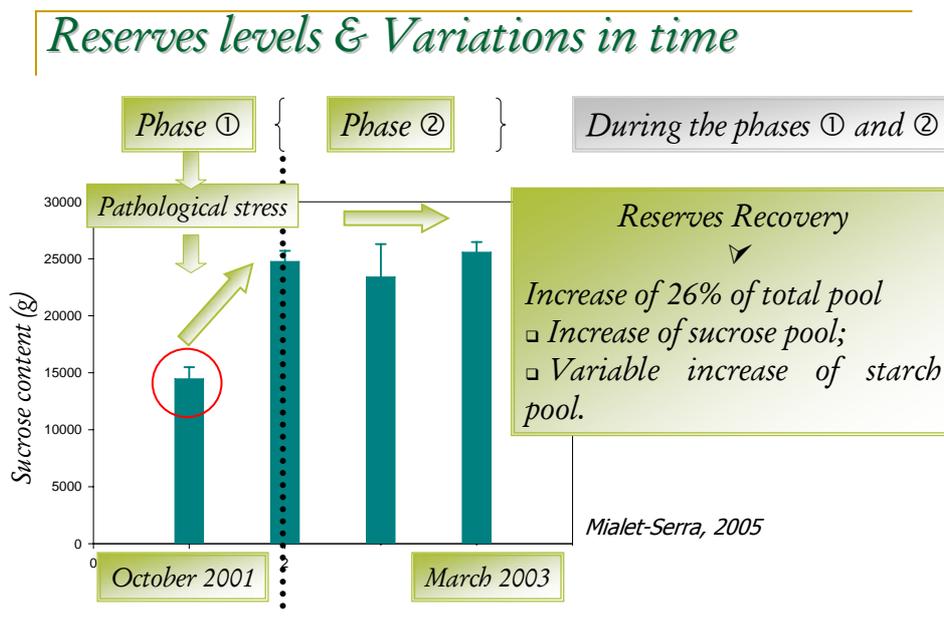


Figure 2- Variation of sucrose content (g) during phases ① & ② in the whole adult coconuts.

- *Phase*②: Reserves variations were weak. This is the case for our two experiments from March 2002 (figure 2). The stability of the reserves pool in the stem, in particular, is notable whatever the treatment applied. This compartment contains reserves little mobilized under our conditions of culture, but which could be mobilizable under more difficult conditions. When the conditions of vegetative growth remain favourable, the storage capacity of tissues could be limited by their anatomy, which would explain a relation between stem growth and reserves constitution, the tank extension becoming necessary to storage.
- *Phase*③: a more “functional” phase in relation with the plant growth rhythm. On the petioles, the total sugars levels remained high during a great part of our observations, and then fall from March 2003 (figure 3). This observation would be to put in relation to the significant increase of fruit loading observed in May 2003.

Reserves levels & Variations in time

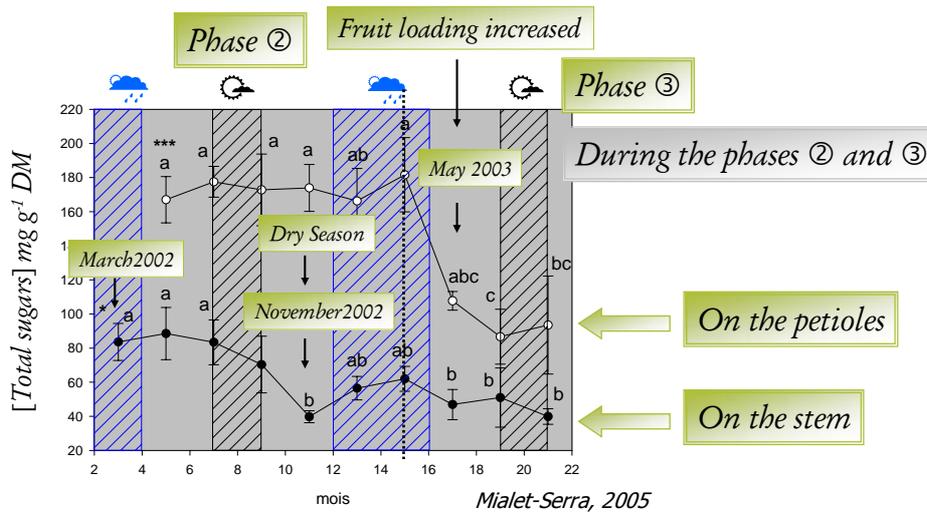


Figure 3- Variation of TS concentrations ($\text{mg g}^{-1} \text{DM}$) during phases 2 & 3 in the stem and the petiole of adult coconuts.

Oil Palm – The situation was more complex than for coconut. It was noted on control treatment (figure 4) that:

- At the whole plant scale, total reserves pool decreased during periods of low radiation and low rainfall;
- A strong inter annual variability was observed, in particular in 2005. This strong decrease remained still unexplained;
- In 2003 and 2004, periods of low reserves level coincided with periods of strong fruit demands (in term of fruit growth, growth and maintenance respirations). In 2005, situation differed.

Reserves levels & Variations in time

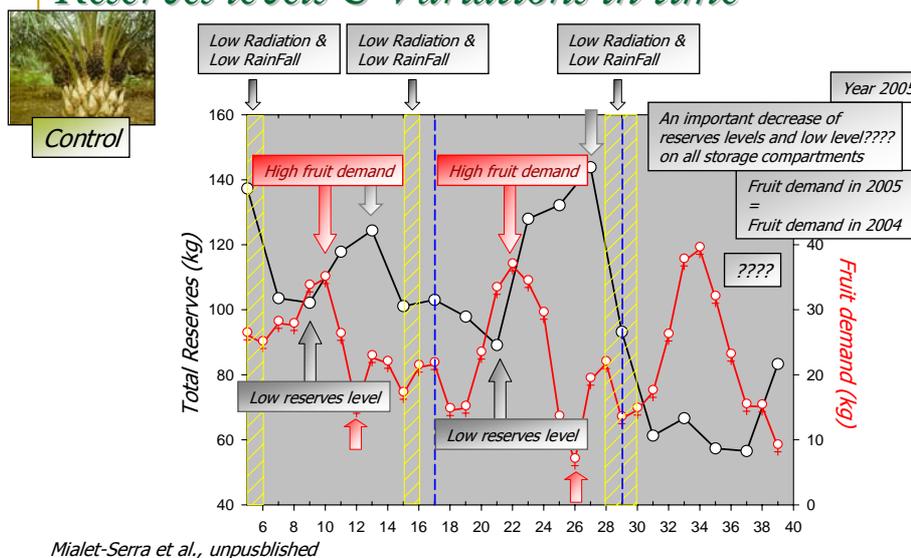


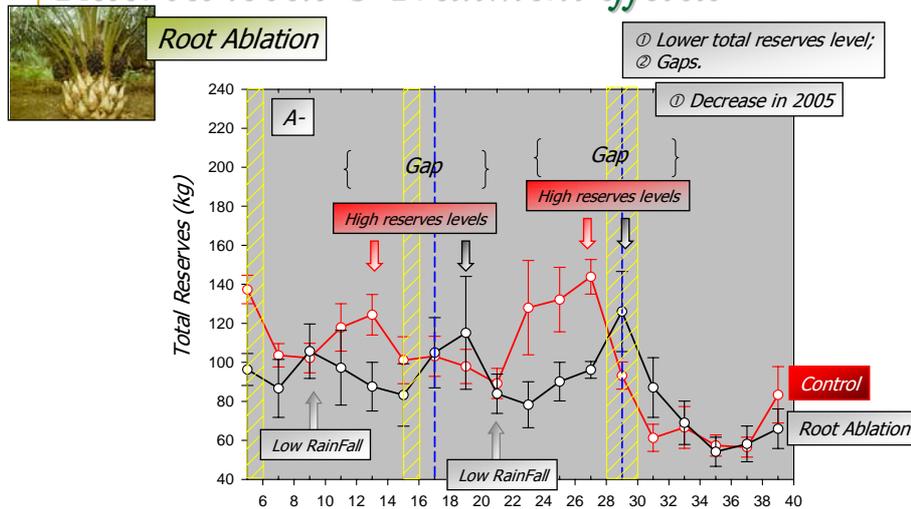
Figure 4- Variations in time of total reserves pool and fruit demands at the whole plant scale on adult oil palms.

Applied treatments effects – For *coconut*, this pool did not vary with the applied treatments; it did not seem to intervene in metabolic balances. For *oil palm*, the situation was complex. But we noted that:

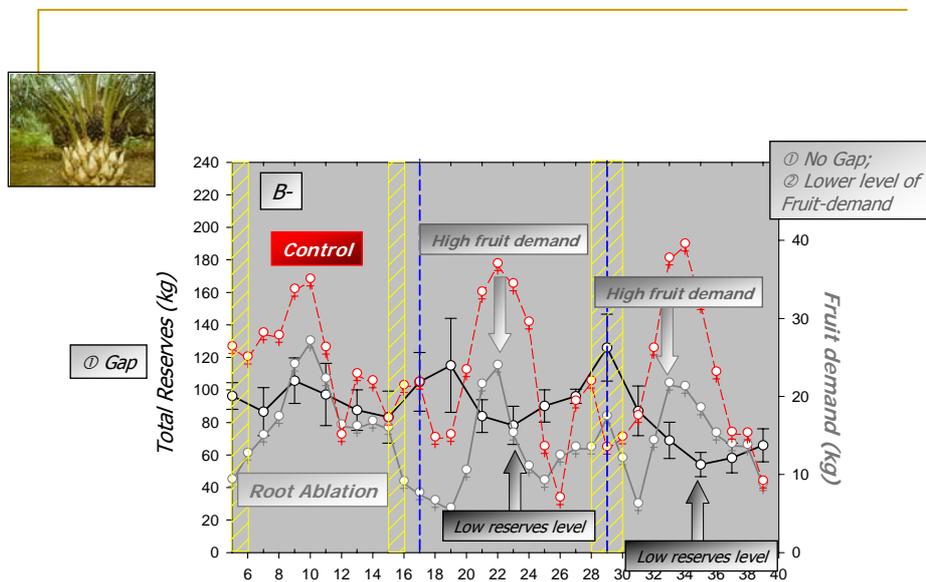
- At the whole scale of plant, total reserves pool was significant higher on bunch ablation treatment; significant lower on "severe leaf pruning" and on "root ablation".
- Gaps were observed between maximum reserve levels on control, and on "severe leaf pruning" or on "root ablation" (figure 5A). On "severe leaf pruning", these gaps could be partially explained too by observed gaps for fruit demands, but not on "root-ablation", because in this precise case, no gaps for fruit demands existed between root ablation and control (figure 5B).

Now, it will be necessary to go into the matter too closely for oil palm.

Reserves levels & Treatment effects



Mialet-Serra et al., unpublished



Mialet-Serra et al., unpublished

Figure 5 – Variations in time and effects of applied treatments (i.e. "Root ablation" and control) (A) on total reserve pool (kg); (B) on fruit demands [fruit growth + (growth+ maintenance) respirations] (kg)

3- Roles

We were gone more in depth for *coconut* to determine and describe carbon reserves roles.

At the storage compartment scale (stem and petiole) on control treatment, we compared variations in time of carbon supply (photosynthesis), total plant demands² (vegetative and fruit demands) and storage/mobilization periods. We observed (figure 6):

- A good convergence between the supply simulated by our model "EcoPalm" (described in these proceedings (Combres *et al.*) and measurements made by Roupsard *et al.* (2006) on the plantation scale;
- An increase of carbon supply during wet season, an increase of total plant demand just after. A gap existed between periods of high supply and periods of high demand.
- A logical consequence of previous observations i.e. mobilization phases. Indeed a first phase was observed on the stem between months 7 and 12; a second phase before the dry season of 2003, intense on both, petioles and stem, which coincides with the period of strong imbalance between supply and demands.

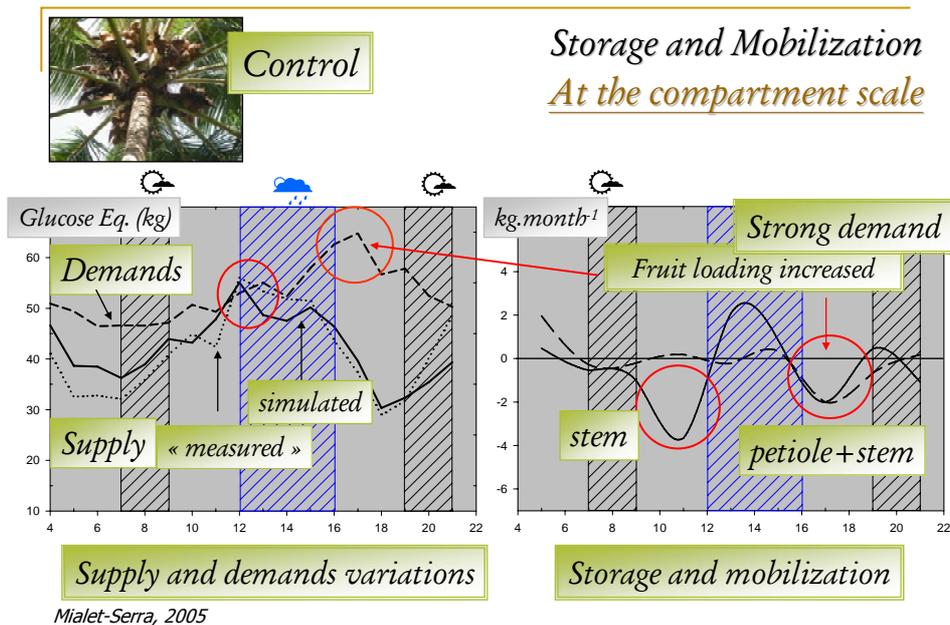


Figure 6- Variations in time of carbon supply (measured and simulated) and of plant demands (left) and of mobilization and storage phases in stem and petiole compartments (right) on control treatment, on coconut.

At the scale of the whole plant on control treatment, total reserves pool variations compared with the balance [supply-demands] (figure 7). This balance represented the part of total demand uncovered by photosynthesis and which, according to logic, would be covered entirely by reserves at this moment.

- For this main reason, these two curves "reserves pool variations" and "supply-demand" should be superimposed. But this was never the case.
- In the case of reserves were never mobilizable, the "supply-demand" curve should be close to zero. However it was never the case. This curve was always lower than zero.

We concluded that systematic distortion seemed to exist for the supply calculation, or both (for supply and demands calculations). Our hypothesis was that the supply would be biased. Why? Its calculation was based on uncertain variables values (rate of radiation interception, conversion rate into assimilates...) and, also, on uncertain factors of corrections which ignored treatments effects on the rate of photosynthesis with lack of *in situ* photosynthesis measurements.

We noted that the supply of carbon simulated by *EcoPalm* was systematically under-estimated on the control treatment. During the first ten months, "supply-demand" and "reserves variations pool" curves

² Total plant demands (g or kg CH₂O) = (vegetative and fruit growth) + (vegetative and fruit growth respiration) + (vegetative and fruit maintenance respiration).

were not in phase (figure 7). The two curves were never superimposed. Differences between these two curves were sometimes very strong, in particular from month 15 (March 2003): Absolute values of [supply-demand] were much higher than reserves variations. So at this moment, we had probably a very important undervaluation of the supply in comparison with the plant demand.

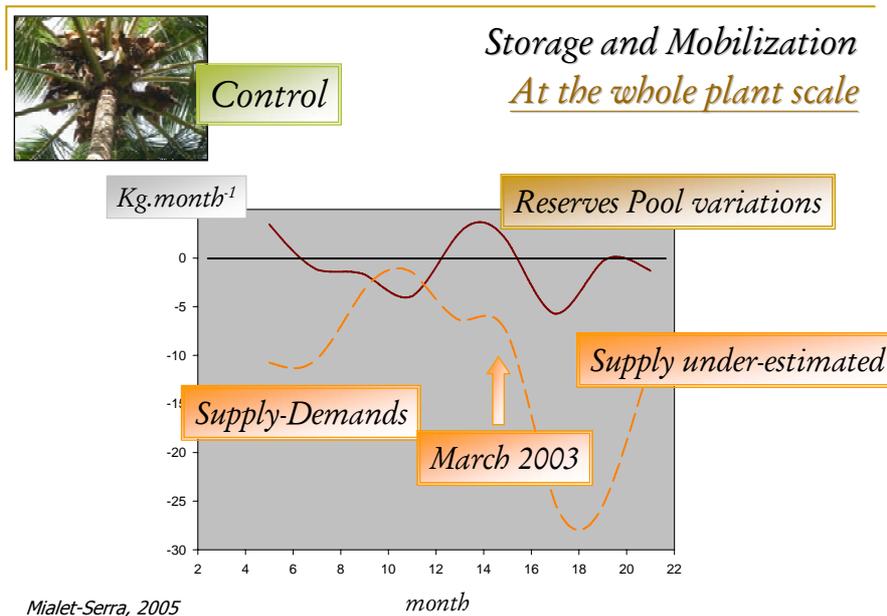
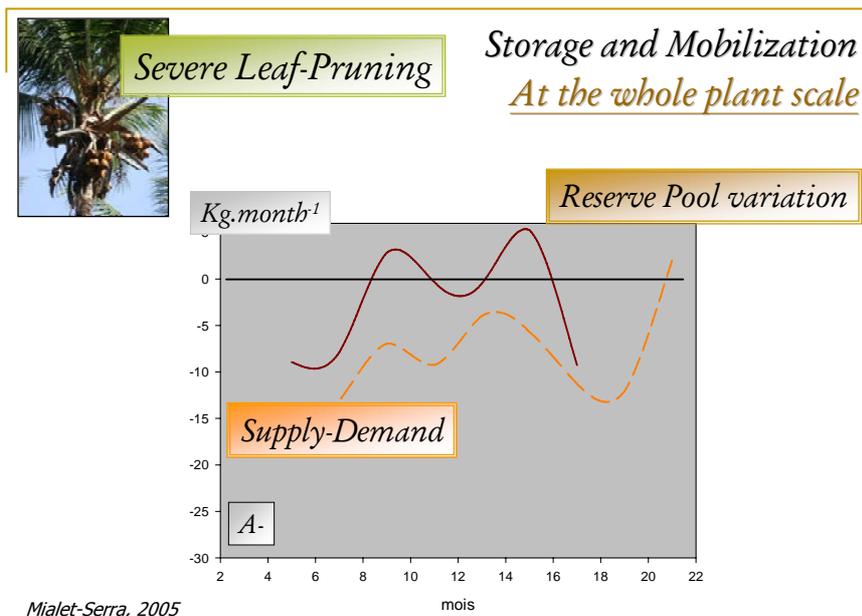
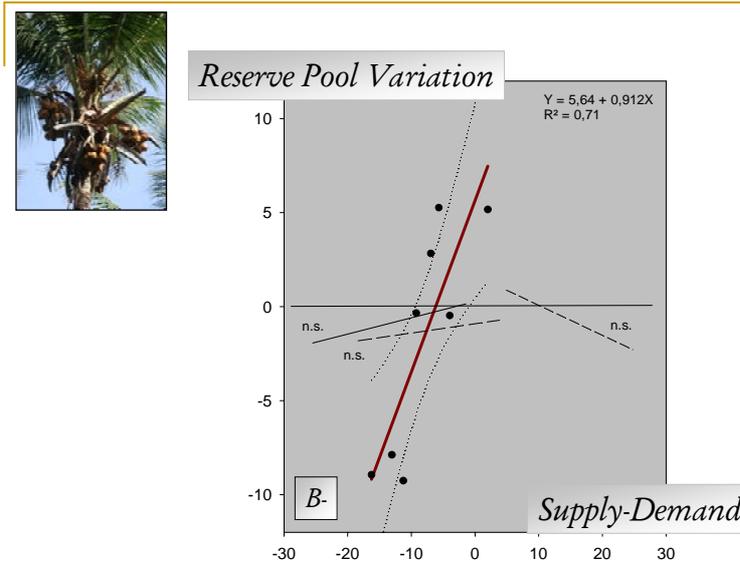


Figure 7- Variations in time of "reserves pool variations" and "supply-demands" on control treatment on coconut at the whole plant scale.

In conditions of limited carbon assimilation (i.e. "severe leaf pruning treatment"), the two curves "reserves pool variations" and "supply-demand", were almost in phase (figure 8A). In this case, the buffer role which the reserves can play was established in particular when photosynthesis is insufficient to cover plant demand. The linear regression obtained on this treatment illustrated this compensation (figure 8B).





Mialet-Serra, 2005

Figure 8- (A) Variations in time of "reserves pool variations" and "supply-demands" on "severe leaf pruning" treatment on coconut at the whole plant scale, (B) linear relation between "reserve pool variations" and "supply-demand".

This analysis showed that feedbacks of demands on supply seemed to exist in coconut. This adjustment was probably very important in a situation of low demand (i.e. Bunch ablation); Localized (in time) in the case of strong fruit demands (i.e. control treatment) and with authorized physiological limits of the plant.

Under our study conditions, the reserves did not seem to play a role in the regulation of the nuts production. The production was variable. The number of fruits per bunch would be controlled early during two stages, at the moment of the differentiation of the flowers and the beginning of nuts growth. The reserves did not seem to have effects on balances which controlled this regulation. Thereafter, the growth of nuts seemed not limited by the sugar availability.

Its role remained secondary. The carbon reserves never supported alone the plant growth, as opposed to temperate trees.

The plant used three mechanisms to balance its daily carbon balance:

- An adjustment of the reproductive demand by an adjustment of the number of female flowers and a reduction of their number by a natural immature nut pruning;
- An adjustment of the photosynthetic rate within physiological limits to specify;
- With storage and mobilization of temporary reserves.

Perspectives

In term of application and in medium term, this study will allow, for *genetic improvement*, identification of interest characters; in *agronomy*, the development of tools of physiological diagnostics and tools of seasonal yield forecast.

Our study present limits. It appears obvious that conditions of cultures constantly favourable limit the explanation of the reserves role in these plants, even if these conditions were changed artificially through the treatments applied. Our work concerned only one ecotype.

In order to consolidate the advanced hypothesis, it would be relevant to be under conditions of more strong and regular constraints and to compare various plant materials.

So it is necessary

- *to widen our experimental base*, as well as possible to exploit genetic diversity and the diversity of the great agro-ecological situations in order to develop a solid base to release some selection adapted to each situation;
- *to simplify and expand our investigations tools* by reducing the initial sampling protocol, by following status changes, and more or less intense carbon flows and by evaluating storage sinks force, by checking hypothesis of feed-back between photosynthetic supply and demand;
- *To enrich EcoPalm* by refining the concepts to describe the management of new-synthesized and stored carbon.

On *coconut*, for the moment, these studies do not continue with fault of facilities in the field and partners. On *oil palm tree*, a new research project (under the large project "EcoPalm") started one year ago. The aim of this new research project is *to characterize carbon management in oil palm* for different genotypes and weather conditions. Measurements of carbon assimilation (photosynthesis...), sugar storage (sugar analyses and main carbon metabolism enzymes activities) and carbon allocation (vegetative and fruit growths, dry matter allocation) are realized in Indonesia, on the experimental plantations of SMARTRI (PT Smart Tbk.), on two sites: without (Sumatra) and with (South Kalimantan) water stress, on two progenies: presumed resistant to water stress (progeny 63) and not resistant to water stress (progeny 83), on two applied treatments (Control and « Bunch-Ablation » to test hypothesis of feedback).

Acknowledgements. CIRAD would like to thank SMARTRI (PT Smart Tbk.) and VARTC (the Vanuatu Agricultural Research and Training Centre) for their logistic and technical support, without which these studies could not have been undertaken.

References

- Croisetu, Murielle, 2003 - Anatomie des différents organes du cocotier (*Cocos nucifera* L.) et localisation des réserves carbonées. Mémoire de stage, UMR BEPC. Maîtrise de Biologie Cellulaire et Physiologie Option Physiologie Végétale Appliquée. 51 p + annexes.
- Fougerolles, Thomas, 2004 – Anatomie des différents organes du palmier à huile (*Elaeis guineensis*) et localisation des réserves amylacées. Mémoire de stage, UMR BEPC. Maîtrise de biologie cellulaire mention physiologie végétale appliquée. Université Montpellier II, 26 p. + annexes.
- Legros, S., Mialet-Serra, I., Caliman, J-P., Clément-Vidal, A., Siregar, F., Widiastuti, L., Jourdan, C. and Dingkuhn, M., 2006 – Carbohydrates reserves in 9 years old oil palm: Nature, Distribution and Seasonal changes, Communication IOPC 2006, Bali, 19 – 23 June 2006.
- Mialet-Serra, I., 2005 – Rôle et gestion des réserves carbonées face à la variabilité du climat chez une monocotylédone arborescente, le cocotier (*Cocos nucifera* L.) : Analyse et bilan. Th. : Ecophysiologie : ENSA de Montpellier, 112 p. + 6 annexes.
- Mialet-Serra I., Clément A., Sonderegger N., Rouspard O., Jourdan C., Labouisse J-P. & Dingkuhn M., 2005 - Assimilate storage in vegetative organs of coconut (*Cocos nucifera* L.). *Experimental Agriculture*, 41 (2): 161-174.
- Rouspard, O., Bonnefond J-M., Irvine, M., Berbigier, P., Nouvellon, Y., Dauzat, J., Taga, S., Hamel, O., Jourdan, C., Saint-André, L., Mialet-Serra, I., Labouisse, J-P., Epron, D., Joffre, R., Braconnier, S., Rouzière, A.,

Navarro, M., Bouillet, J-P., 2006 – Partitioning energy and evapo-transpiration above and below a tropical palm canopy. *Agricultural and Forest Meteorology*, 139: 252-268.

Siregar, F., 2005 (*in press*) – Analysis of vegetative and fruit growths of adult oil palm: seasonal changes and applied treatments effects, ATP training report. 7p.



COCONUT FRUIT QUALITY ASSESSMENT THROUGH MATURATION

By Alexia PRADES, Isabelle MIALET-SERRA, Fabrice DAVRIEUX, Anne CLEMENT-VIDAL

CIRAD, RU 33 "Quality of tree crop products", Montpellier, France; CIRAD, RU 59 "Integrative modeling", Montpellier, France.

Introduction

Coconut fruit has always been more than a simple oil source for millions of persons. Although copra (the dried kernel of coconut fruit) is the more common marketable product on international trading place, many others products are available on local markets. Some of these products such as desiccated coconut, coconut milk or coconut water (juice of the young nuts) are even commercialised in huge volumes, especially in Asia and South America.

Coconut tree selection and breeding has always been interested in increasing copra yield, whereas there is a need today for more diversified selection schemes. Coconut growers do not only ask for varieties with high copra yield but also for various variety for sweet and thick kernel or sweet coconut water.

But little is known on the changes of fruit quality at different development stages or ages. The aim of this specific survey was to study the elaboration of the fruit quality to improve our knowledge on yield elaboration, in terms of quality. The expected results were supposed to lead to the selection of good quality predictors thus to improve the coconut culture management *in situ*. Finally, linking the quality criteria variations to the dynamic of carbohydrates reserves would help to build the future model of the coconut plant trophic status.

The global strategy to attend these objectives was based on four hypotheses:

- H0 (preliminary hypothesis): there is no gradient into one bunch at one stage of maturity for any of the measured criteria
- H1: fruit quality differs at each stage of development
- H2: quality can be predicted thanks to few indicators
- H3: fruit quality level is related to trophic status of the plant via « source-sink » phenomena

Material and Methods

Plant material

The Vanuatu coconut hybrid, a cross between Vanuatu Red Dwarf, VRD and Vanuatu Tall, VTT was studied. Its acronym is "VRDxVTT". The orchard we used was the same as for the carbon storage survey: located on VARTC Research Station, Santo, Vanuatu. Coconut trees were 17 years old at the beginning of our study.

Methods

For H0, 6 trees were harvested at rank 20 and rank 22, which corresponds to coconut fruit aged of 9 and 11 months. Fruits were collected from three positions into the bunch: S (apical part of the bunch), M (medium part of the bunch), B (base of the bunch). The harvest was done in April 2003. On each of the three fruits harvested, 22 criteria were measured and calculated (Table 1).

For H1, H2 and H3, 10 coconut trees were selected at random on the same orchard. The survey lasted 3 years between 2001 and 2003. On each coconut tree, five ranks were harvested : rank 14, 17, 20, 22 and 24 corresponding to coconut fruit respectively aged of 3, 6, 9, 11 and 13 months. Three fruits per bunch were collected and measured for the same criteria as for H0 (Table 1).

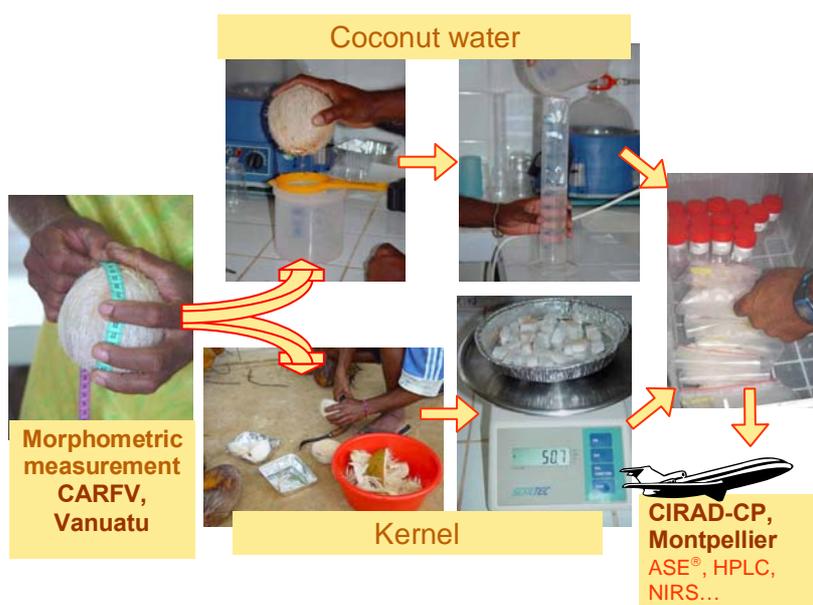
Table 1- List of usual and new measured criteria on the coconut fruit

| <i>Morphometric criteria</i> | <i>Method</i> | <i>Part of the fruit</i> |
|---|--|--|
| Polar and equatorial circumferences (mm) | Ribbon (1meter) | Fruit, nut |
| Fresh weight (g) | Balance (0.001g) | Fruit, nut, husk, shell, kernel, water |
| <i>Volume (ml)</i> | Test tube | Water |
| <i>Thickness (mm)</i> | Calliper | kernel, shell |
| <i>Biochemical criteria</i> | | |
| Dry matter (g/100g) / moisture content (g/100g) | Oven at 70°C (husk and shell) or freeze-dryer (kernel and water) | Husk, shell, kernel, water |
| Brix (refractometer) | Manual refractometer | Water |
| pH | pHmeter | Water |
| Oil concentration (g/100gDM=%db) | Automatic Solvent Extractor, ASE® | Kernel |
| Total soluble sugars, TSS (g/100gDM=%db) | HPLC | Kernel, water |
| Sugars and polyols profile (g/100gDM) | HPLC | Kernel, water |
| <i>Calculated criteria</i> | | |
| Copra (g) | | Kernel |
| Empty nut weight (g) | | Nut |
| Shape factor = Eq. Circumf. / P. Circumf. | | Fruit, nut |
| Sweet taste Index : I.S. | | Kernel, water |

For H1 to H3, our methodology was as followed:

- o Selecting new criteria to measure on fruit bunches with focus on carbohydrates and lipids
- o Measuring these criteria during fruit development (Picture 1)
- o Looking at relations among criteria to find efficient quality predictors
- o Studying « leaf-bunch-fruit » relations through carbohydrates flux

Some of the measurements such as circumferences, weights, moisture content, Brix, pH were performed in Vanuatu. Others criteria such as oil concentration, total soluble sugars, sugars and polyols profile were performed on freeze-dried samples sent by plane to Cirad, Montpellier, France.



Picture 1- Description of the method of sampling

Results

H0: There is no gradient into a coconut bunch for quality criteria of the fruit

An analysis of variance was performed on the data collected from the six trees. There were no significant effect ($p=0.05$) of the fruit position on the bunch on any of the 22 measured or calculated criteria (examples in Figure 1). This result implied that we could reduce our sampling method and collect only 1 or 2 fruits per bunch to assess the fruit quality during maturation. It was considered as an important result as samples were analysed in France whereas harvested in Vanuatu and a negative result would have considerably increased the number of samples, thus the cost and duration of the study. Furthermore, although this question seemed to be fundamental before studying fruit quality on coconut bunches, we did not find any answer to it in the literature.

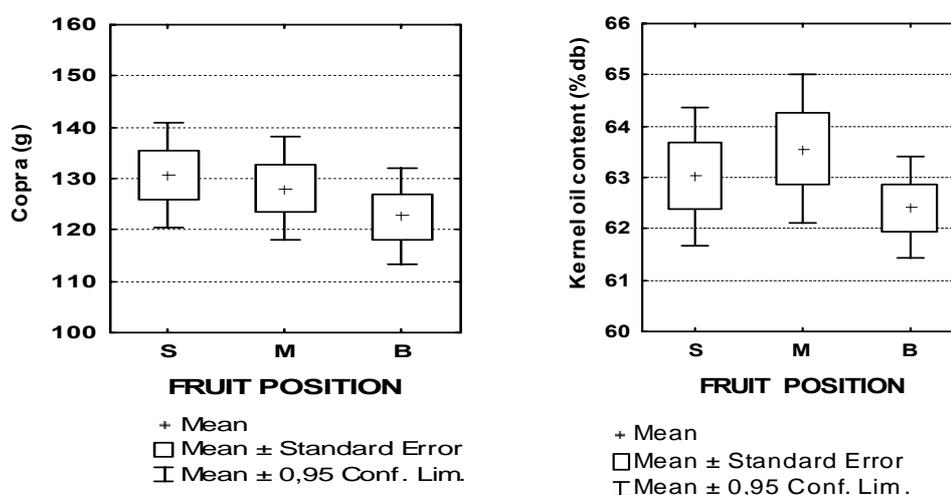


Figure 1- Variations of copra (g) and kernel oil content (% dried matter) of coconut fruit according to the fruit position into the bunch at rank 22, VRDxVTT, Vanuatu

Answer to H0: there is no gradient into one coconut fruit bunch at the age of 9 or 11 months for VRDxVTT hybrid growing under Vanuatu climatic conditions for any of the measured criteria.

H1: The quality of the fruit differs at each stage of development

The analyse of the results of the 22 measured criteria on the coconut fruit aged of 9 to 13 months lead to various conclusions.

For morphometric criteria, we found that the fruit equatorial circumferences stabilised at rank 18 and global nut shape also stabilised at rank 17. As the study was conducted on several harvest period, we analysed the effect of a wet or dry harvest period on the criteria. No significant effect ($p=0.05$) of the harvest period was found neither on the shape of the fruit nor on the nut. Concerning the fresh weights of the fruit, we distinguished two phases. The first one is before rank 17 where the growth was very rapid and the second one is after rank 17 until rank 24 (mature fruit) where the fresh weight regularly decreased (Figure 2). This evolution was mainly due to the variations in the husk fresh weight. Kernel appeared between rank 14 and 17. Fresh and dry weights of kernel and shell stabilised from rank 20. The maximum fresh water weight was around rank 17, age of 6 months.

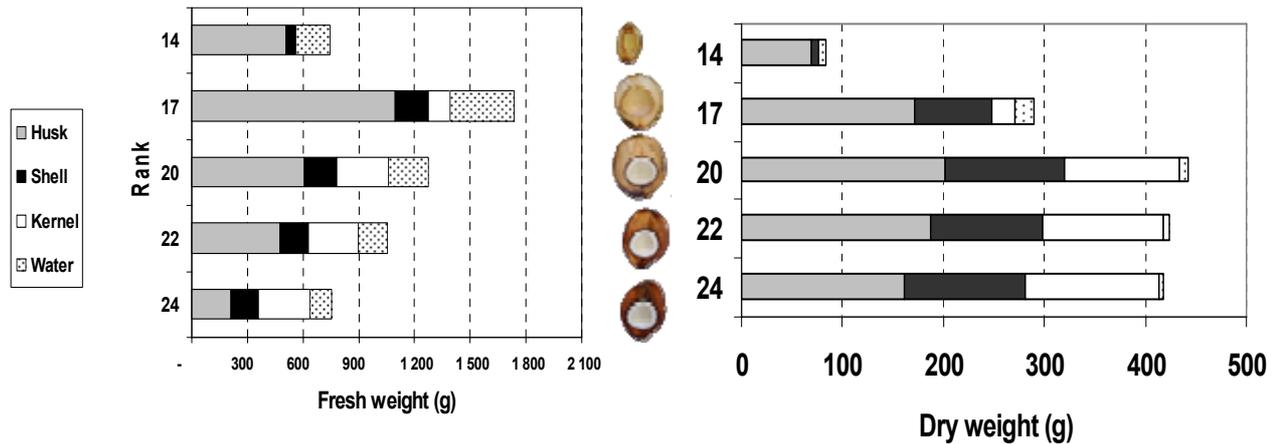


Figure 2- Evolution of the fresh and dry weights (g) of the 4 parts (husk, shell, kernel and water) of coconut fruits between rank 14 and 24, VRDxVTT, Vanuatu, April 2003

For biochemical criteria, kernel oil concentration and total soluble sugars (TSS) showed antagonistic trends (Figure 3). Both were negatively correlated, confirming that soluble sugars may be the precursors of the lipids in the kernel. We did not noticed any effect of the harvest period on these two criteria. When we analysed the sugars and polyols profile, sorbitol was found to be one of the major carbohydrates of the kernel and the water, after sucrose and before fructose and glucose, in terms of percentage.

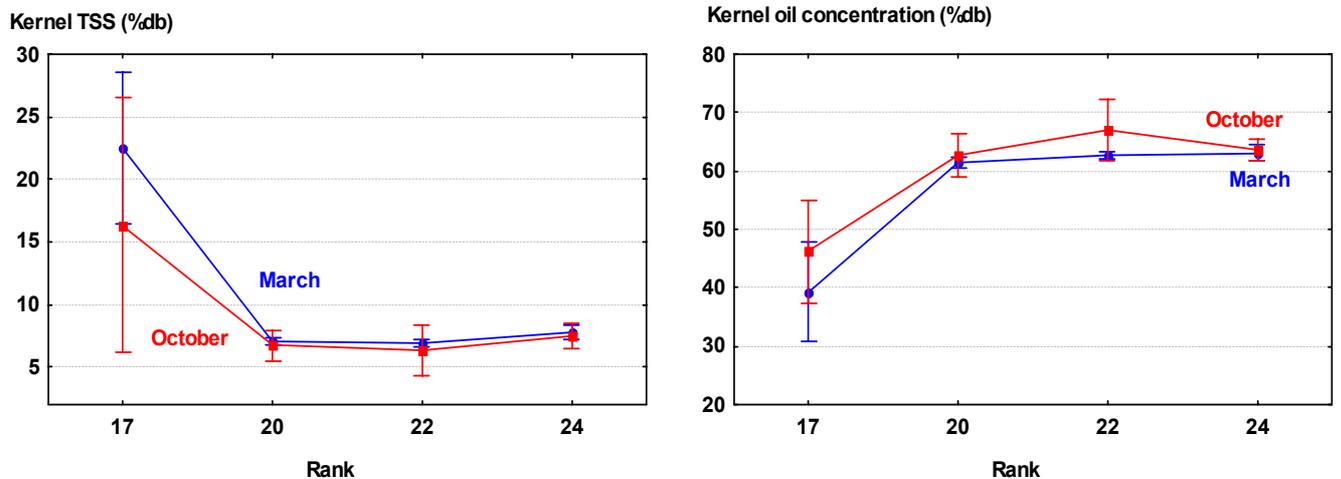


Figure 3- Evolution of the total soluble sugars (TSS) and oil concentrations (% dried matter) of the kernel of coconut fruits between rank 17 and 24, VRDxVTT, Vanuatu, April 2003

Answer to H1: there were global changes of the measured quality criteria at each development stages. Some critical steps of the development of the fruit have been detected, especially for coconut shape and the quality of kernel and water. They could help in the determination of the next step of the study concerning quality predictors.

H2: Few efficient indicators might predict the coconut fruit quality

The first interesting criterium found was the equatorial circumferences of the fruit at rank 18. For VRDxVTT hybrid, it could be a good predictor of the grade of the mature coconut fruit as it did not change until rank 24.

Others interesting criteria could be the kernel and shell thickness. Compared to other varieties, VRDxVTT showed a very rapid growing phase of the kernel between rank 17 and 20. For example, the hybrid from Côte d'Ivoire, PB121, on the contrary, showed a slow growing phase between ranks 20 and 24 and did not have any kernel at rank 17. Depending on the variety, the harvesting of the coconut bunches could be optimized and managed differently.

Last example of these indicators is the sweet taste index, calculated from the sugars and polyols concentrations. This index gave us a good idea of the actual sweet taste of the kernel and the water and could help producers or breeders to select varieties with high potential value for coconut water drink market.

Finally, a matrix of correlation of the previous results within criteria and within stages of development helped us to select some indicators that could be of interest to predict fruit quality. For examples, i. nut fresh weight was correlated to kernel fresh weight, ii. fruit equatorial circumferences at rank 20 was correlated to nut fresh weight and to water volume, iii. fruit fresh weight was correlated to nut fresh weight at ranks 20, 22 and 24. All these indicators could be combined to classify the raw material at harvest. It might help the producers to select the right raw materials for the right processors. A desiccated coconut factory will not have the same demand as a copra manufacturer.

Answer to H2: Yes we found some efficient indicators and predictors of the coconut fruit quality on the VRDxVTT hybrid in Vanuatu. Many of them are simple to measure and might help producers to classify and grade their production. They could also help breeders to "reevaluate" the coconut trees biodiversity. Furthermore they could help to predict a quantitative and qualitative yield in a mathematical model.

H3: fruit quality level is related to trophic status of the plant via « source-sink » phenomena.

This stage is currently in progress. The aim of this part of the study is to link our results to the heart of this ATP: carbohydrates reserves and trophic status of the tree. The questions we tried to answer were "Which relations between a frond and its bunch in term of carbon exchange before harvest and their trophic status?" and "Was there any effect of the harvest period on these exchanges?"

After a short analyze of our data, we found only a partial answer to the first question. There could be a relation between total soluble sugars concentration of the leaf at rank 9 and the number of fertilized fruits on the bunch. This result could be the beginning of a quantitative yield predictor and could be further integrated to the model.

Answer to H3: we did not find any simple relationship between sugars concentrations in a frond and its corresponding bunch but data are still to be examined.

Conclusion and future

The quality of coconut fruit was assessed during "on tree" maturation in order to find new descriptors and predictors of the yield of the coconut tree. These new predictors were supposed to be related to the trophic status of the plant and more especially to the carbon storage levels in organs such as fronds.

Many interesting results were found in the first part of the study: new efficient descriptors of the quality, new predictors of the yield. But all these results were found on one variety and it is not enough to say that they are robust and adapted to all coconut varieties. We should say here that the

same type of survey was conducted, during the same period, on the hybrid PB121 of Côte d'Ivoire. Results of both studies confirmed the relevance of some of the selected criteria but others have still to be tested on other varieties and under various climatic conditions.

The links between "leaf-bunch-fruit" are still to be explored. It seemed that the rather good climatic conditions of the Vanuatu did not emphasize enough the variations of sugar concentrations in the different organs. It could therefore be a good challenge to look at these phenomena on other varieties such as dwarf coconut trees and under more severe conditions such as drought.



DEVELOPMENT AND USE OF ORIGINAL ANALYTICAL AND INVESTIGATIONS TOOLS

**RESERVES POOLS IN VEGETATIVE AND FRUIT COMPARTMENTS OF COCONUT AND MANGO TREE:
ANALYSIS BY NEAR INFRARED SPECTROSCOPY (NIRS)**

By Fabrice DAVRIEUX, Isabelle MIALET-SERRA, Alexia PRADES,
Anne CLEMENT-VIDAL & Frédéric NORMAND.
CIRAD, Montpellier, France

Introduction

More than 5000 samples were systematically harvested on all vegetative and fruit compartments of coconut and mango tree and analyzed in our laboratories. On this important batch of samples, four determinations were systematically done i.e. the glucose, the fructose, the sucrose and the starch concentrations on all samples and the sorbitol concentration on copra samples. In parallel to the classical tools for analyses (by HPLC and enzymatic methods), near infra-red spectroscopy was tested. High analytical capabilities of NIR spectroscopy made this technique very suitable for rapid biochemical analyses in terms of easy use, cost and reliability (Lachenal, 1998).

This study aimed to evaluate the potential of the NIRS for the determination of the sugar and the starch concentrations in stem and fronds (main storage compartments) and copra samples for coconut and in all vegetative and fruits compartments for mango tree and for a description of variations in time for all compartments followed for the both species. Equations of calibrations were established in order to predict the concentrations of these various compartments on the basis of spectrum. The two following and fundamental questions were addressed: (i) was the NIRS accuracy identical or close to that of classical analyses in laboratory? And (ii) was it possible to make same conclusions or closed conclusions with classical and NIRS methods?

Materials and Methods

Principle of spectra acquisition

The near infra-red spectroscopy is a spectroscopic method whose principle was based on the absorption of the near infra-red radiation (wavelengths between 800 and 2500 nm) by the organic matter.

The method was based on the number of specific chemical bonds as O-H, N-H, C-H types etc. These bonds performed like oscillators which vibrated permanently at different frequencies according to their nature. A particular chemical bond can absorb a near infra-red radiation whose frequency was equal to its frequency of vibration and thus passed from a fundamental state to an excited state. In the same way, the energy of radiations whose frequencies were multiples of the fundamental frequency (called *harmonics*) can be absorbed. In the infra-red zone, absorptions were not due to the fundamental vibrations of the molecules, but to the harmonic vibrations and the combinations vibrations. A wavelength can therefore be linked to a given bond, (i.e. H-OH bond of the water molecule is linked to 1 900 nm wavelength). The quantity of light energy (photons) absorbed follows Beer-Lambert's law.

In the framework of the ATP project, measurements were taken with a sequential FOSS 6500 analyser (where light absorptions were collected sequentially in time) with only one detector, equipped with a FOSS auto sampler taking up to 50 samples.

Three grams of sample were analysed by diffuse reflection from 400 to 2500 nm at 2 nm intervals. One spectrum per sample was recorded (figure 1).

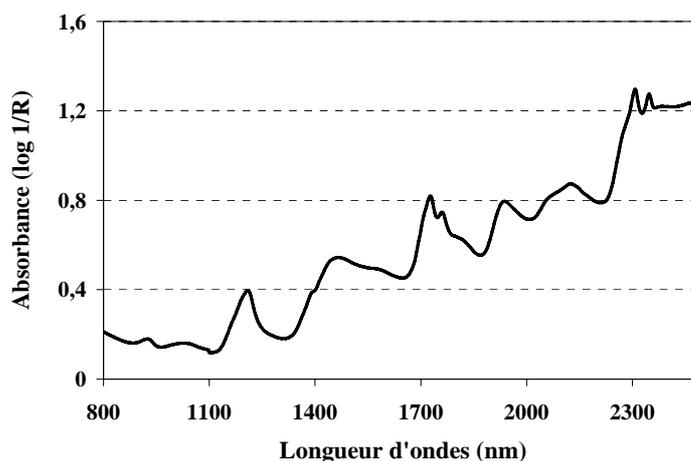


Figure 1- An example of spectrum

Statistical analyses

Step ① - The descriptive statistics of the reference value were computed using Xlstat (2006, addinsoft).

Step ② - Relevant information was extracted from (i) reference values and (ii) the matrix of spectral data by Principal Component Analysis (PCA). Based on the matrix of spectral data, the Mahalanobis H distance from the mean spectrum was calculated for each spectrum. An H distance over 3 corresponded to a probability of less than 0.01 that the sample belonged to this population. Sample selection for the model elaboration was based on these distances; spectra with high Mahalanobis H distance did not be used for the calibration.

Step ③ - The absorbance of a compound was proportional to its concentration (Beer-Lambert law). This law, defined for a simple component, was extended here to a mixture of components. The *predictive model* connecting the values of absorption (given spectral) and the reference values (obtained by HPLC) was a multilinear model:

$$Y = F(X) + E$$

X was the matrix of the spectral data (N samples and p wavelengths).

Y was the matrix of the data obtained in laboratory (N constituent samples and Q).

F was the mathematical model.

E was the term of error (residue).

The method used was the "*partial least squares*" method (PLS). The PLS method was a regression method, to combine the whole X variables (spectral values); this combination was found by maximizing the covariance with y (laboratory values) (Davrieux, 1997). The calibration was done after elimination of spectra from the values of T test (Student test). The limiting value of T is fixed at 2.5 (i.e.: a sample of which the value of $[(y_i - \hat{y}_i)/SEC] > 2.5$ is isolated).

Step ④ - the quality of the model and its accuracy were evaluated with several statistical parameters which were:

- *The determination coefficient (R^2)*: the part of the Y variance explained by the model. When R^2 was close to 1, the model explained well the variable;
- *The standard error of calibration (SEC)*: an estimation of the standard deviation of the random standard error between the values of reference (laboratory values) and the predicted values (index of precision of the calibrations and the adjustment of the predicted values);
- *The standard error of prediction (SEP) and the standard error of cross validation (SECV)*: the precisions with which the calibration model will be able to predict new samples. SECV was calculated on the samples of the population used for the calibration; SEP was calculated on new samples which could be added to the existing base. These two parameters must be close. In order to minimise over-fitting of the equations, cross validation was used during calibration development. This method of cross-validation was based on the prediction of sub-groups (in our case, 4 subgroups were used) by equations developed on the rest of the data base (Thuriès *et al.*, 2005);
- *The Standard Error of Laboratory (SEL)*: the precision of the reference method used in laboratory. It represented normally the standard deviation of analyses obtained on several repetitions (up to 10

repetitions) of the same sample at a given moment (*test of reproductibility*). However in this study, it represented the standard deviation of analyses obtained on several repetitions (up to 7 repetitions) of the same sample treated at regular times (*test of repeatability*). For each plant organ, a reference sample (or internal reference) was selected. It was systematically inserted in a batch of 20 samples for analyzes and followed then the same analytical treatment (from the extraction to the determination of soluble sugars and starch concentrations). For each sugar, a coefficient of variation (CV) is calculated (equal to SEL). The CV value depended on the sugar concentration. Noted that one dilution was made, so it was not possible to have the same precision with a sugar, strongly represented (e.g. sucrose) or with a sugar at the traces state (case of the starch). In brief:

- When sugar concentration was less than 1 mg per g of DM, CV was ranged between 20 and 40%. The reference value was not very precise.
 - Between 1 and 2 mg g⁻¹ of DM, CV was located around 10%; the result was acceptable;
 - From 2 mg g⁻¹ of DM, CV was closed to 5%, the result is precise.
- *The Ratio Performance/Deviation (RPD)*: the ratio between the population variability (standard deviation) and the calibration precision (SECV or SEP). It was often considered that a model was interesting from RPD equal to 3-4 and very good beyond 6 (but this judgement must take account of the population variability and/or the accuracy of reference values or laboratory error).

Plant materials

For *coconut*, 1177 spectra from dry samples (in powder) of leaflets (275), petioles (432), rachis (297) and stem (297) were acquired and all these dry samples were analyzed in laboratory (reference values obtained). These samples were harvested during 3 different years on one hybrid in Vanuatu (South Pacific). 385 coconut kernel spectra (at various maturation stages, on two coconut hybrids, in two different countries (Ivory Coast and Vanuatu), over 3 years) were acquired. Only 128 selected samples of dry kernel were analyzed in the laboratory for total soluble sugars (TSS) and lipid concentrations.

For *mango tree*, 1967 spectra of leaves, inflorescences, growth units (GU), wood, roots and fruits were acquired and all these dry samples were analyzed in laboratory (reference values obtained). These samples were harvested during one year on 13-year-old mango trees, cv. Cogshall grafted onto 'Maison rouge', in an experimental orchard in Reunion Island (21°06'S, 55°32'E) (Normand *et al.*, 2005). A first NIR model was calibrated only on 73 root samples.

Results & Discussion

On coconut stem and fronds compartments

Population Structure

Principal Component Analysis (PCA) applied to spectra data allowed to identify possible structures (groups, family of entities...) (figure 2-). The cluster of points was uniformly distributed according to the first CP which explained 73.6 % of the initial variance (figure 2, on the left). There was three apparent group structures i.e. trunk (or stem), [petiole+rachis] and leaflets (figure 2, on the right and the left). In fact, four populations were defined for calibration i.e. stem, petioles, rachis and leaflets populations but petiole and rachis populations were closer and could have been mixed.

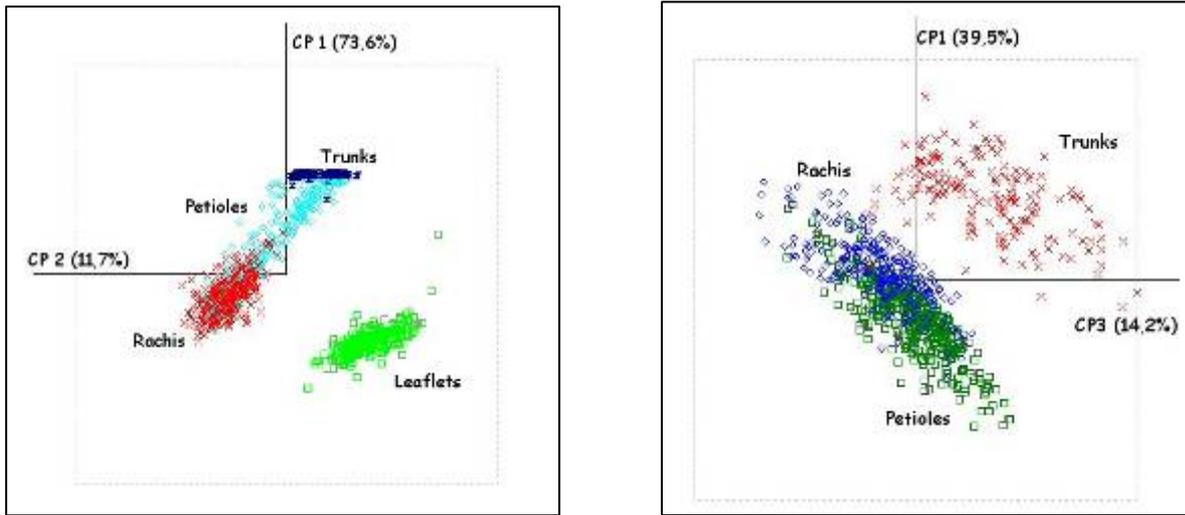


Figure 2 –Representation of the 1177 coconut leaflets, rachis, petioles and trunk (or stem) (on the left) and of the 902 coconut petioles, rachis and trunk (on the right) for the two first principal components

For each population, PCAs were done on the base of all spectra. These iterative procedures allowed to eliminate 4 outlier samples for leaflet, 7 for petiole, 14 for rachis and 4 for stem and to constitute reduced bases for calibration. In second approach, various numbers of principal components (CPs) were preserved on PCA realised on the reduced basis (without outliers) and explained high percent of native variances.

In all the cases, scatter plots of spectra on the two principal axes did not highlight of specific structure according to the sampling years (figure 3, all). However, for starch, a great dispersion of samples according to the first two factorial axes was observed.

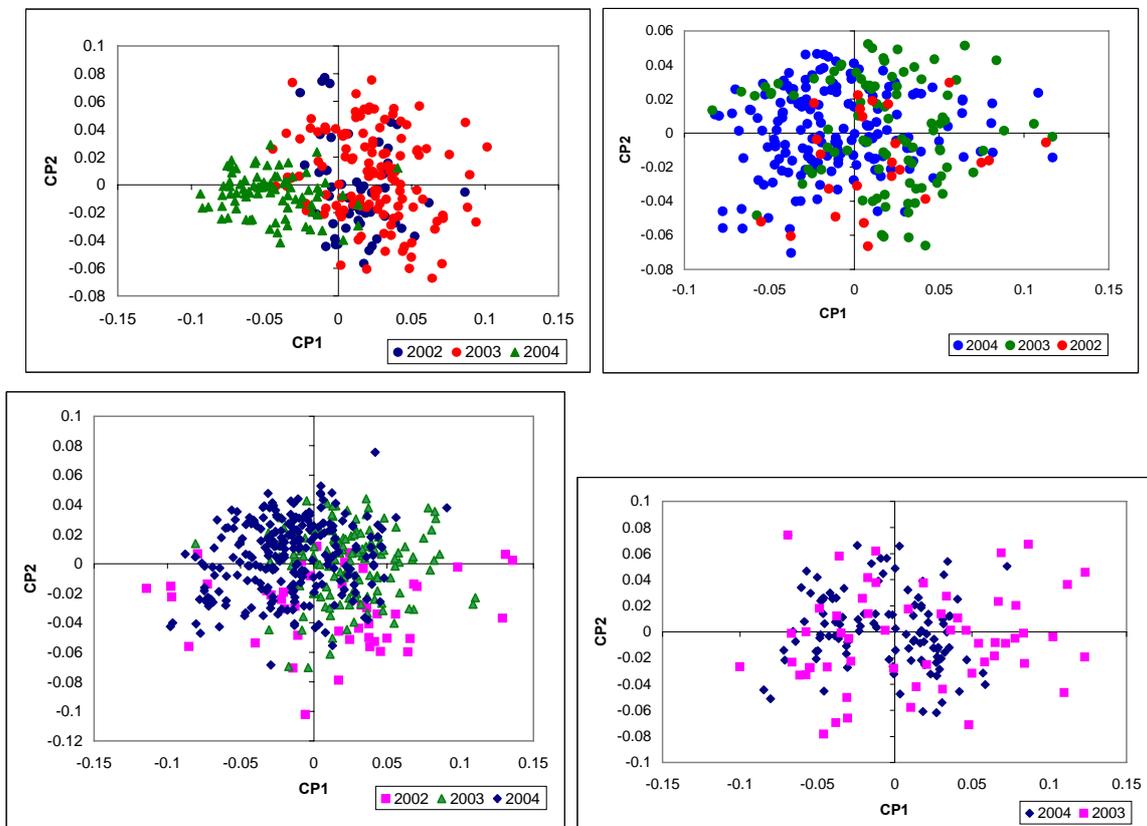


Figure 3 – Representation of the leaflets (left, up), the rachis (right, up), the petioles (left, down) and of the stem (right, down) samples for the two first principal components.
On Leaflets

Descriptive statistic of reference values

The reference values of *leaflets* concentrations were statistical described in table 2-. The distribution of sucrose concentration was bimodal (figure 4): 22.7% of reference values were between 0.2 and 6.2 mg g⁻¹, the major part of values was distributed normally between 24 and 36 mg g⁻¹ (figure 3).

Table 2- Descriptive statistics of the reference concentrations (mg g⁻¹ DM) of leaflets

| | glucose | fructose | sucrose | starch |
|-----------|------------|------------|------------|------------|
| Values Nb | 273 | 273 | 273 | 185 |
| Range | 0.2 – 27.2 | 0.1 – 24.6 | 0.2 – 60.6 | 0.1 – 13.5 |
| Mean | 10.0 | 9.5 | 23.4 | 3.6 |
| SD | 7.3 | 6.8 | 14.0 | 2.8 |
| SEL | 4.8 | 5.3 | 4.9 | 7.4 |

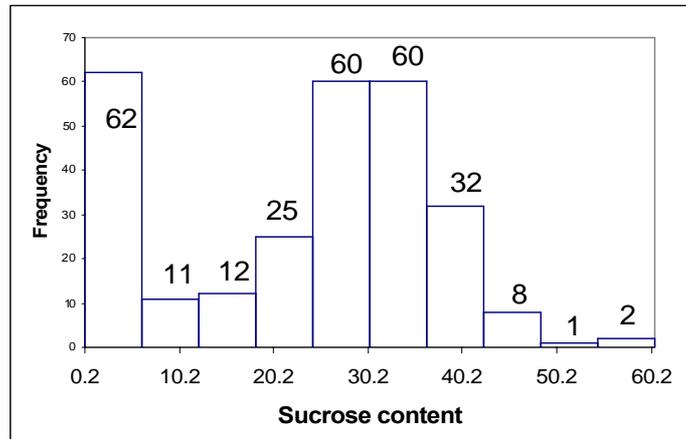


Figure 4 - Distribution of the sucrose concentrations in the leaflets

The distribution of the starch concentration was strongly dissymmetrical on the left (figure 5); 69.7% of values were distributed between 0.1 and 4.1 mg g⁻¹. The distributions of the glucose and fructose concentrations were rectangular: 13% of fructose values were distributed between 0.9 and 2.6 mg g⁻¹ and 29% between 2.6 and 5.0; for glucose 43% of the values were distributed between 0.2 and 5.6 mg g⁻¹.

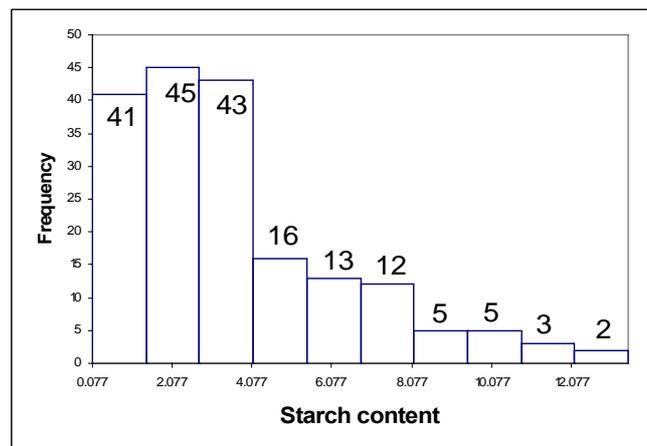


Figure 5 - Distribution of the starch concentrations in the leaflets

NIRS Calibration and analysis of predicted values

The calibrations developed for *fructose*, *glucose* and *sucrose* were powerful (R² = 0.96, 0.97 and 0.95; RPD higher than 3 (table 3 & figure 6)).

Table 3- Statistical parameters of each sugar NIRS equations for leaflets

| | N | Moyenne | SD | SEC | R ² | SECV | CV (%) | RPD |
|----------|-----|---------|------|-----|----------------|------|--------|-----|
| glucose | 253 | 10.1 | 7.2 | 1.1 | 0.97 | 1.6 | 16 | 4.4 |
| fructose | 266 | 9.6 | 6.8 | 1.4 | 0.96 | 2.0 | 20 | 3.4 |
| sucrose | 261 | 23.3 | 13.5 | 2.9 | 0.95 | 4.0 | 17 | 3.4 |
| starch | 181 | 3.5 | 2.7 | 1.3 | 0.76 | 1.6 | 45 | 1.7 |

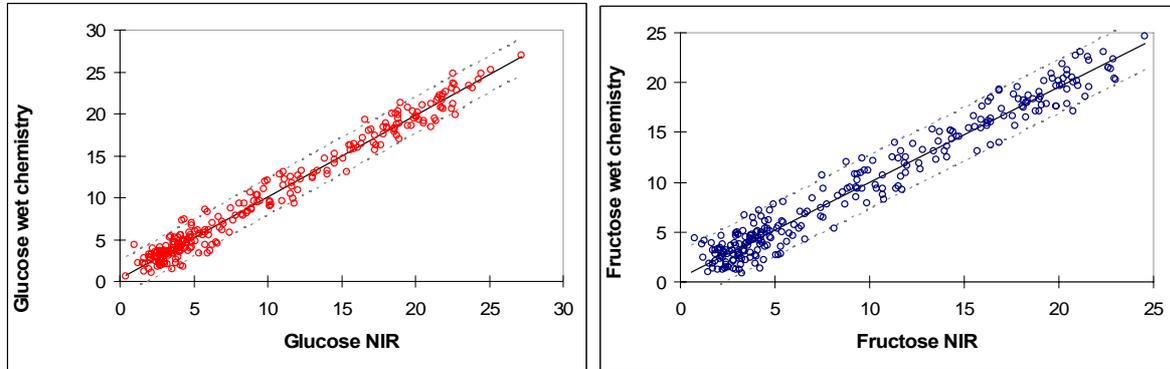


Figure 6- Correlations between predicted values (NIR) and reference values (laboratory or wet chemistry) for Glucose (left) and Fructose (right).

For *sucrose*, the scatter plot on figure 7 showed the two populations described previously in figure 3-. Specifics calibrations (i.e a calibration on only one population with concentrations > 20 mg g⁻¹) did not improved the model.

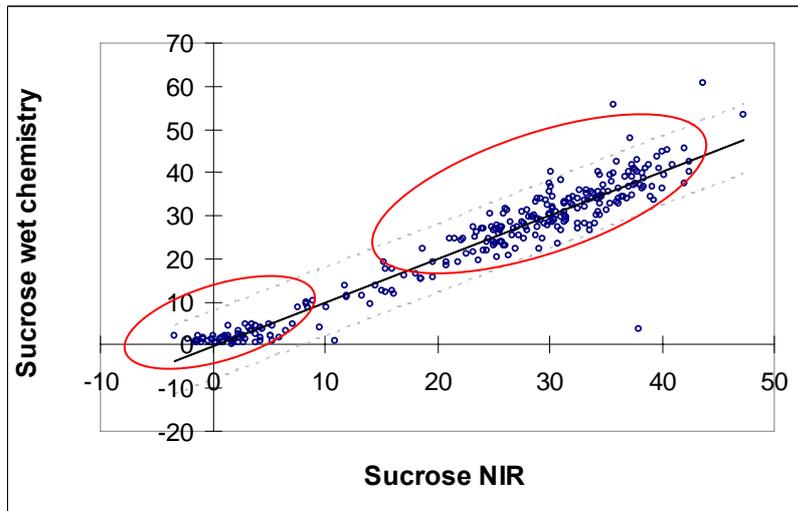


Figure 7- Correlations between predicted values (NIR) and reference values (laboratory or wet chemistry) for *sucrose*.

The equation developed for the starch concentration was less powerful ($R^2 = 0.76$, $RPD = 1.7$, $NIR\ CV = 45\%$, figure 8). Highest residue values were observed for low content values (< 5 mg/g) (figure 9). These results can be explained by a dissymmetrical distribution of these concentrations (70% were lower than 4 mg g⁻¹, see figure 4) and by a greater inaccuracy of the determination in laboratory linked to these low starch concentrations in coconut in general, in particular in this frond compartment wet chemistry CV = 7.4%).

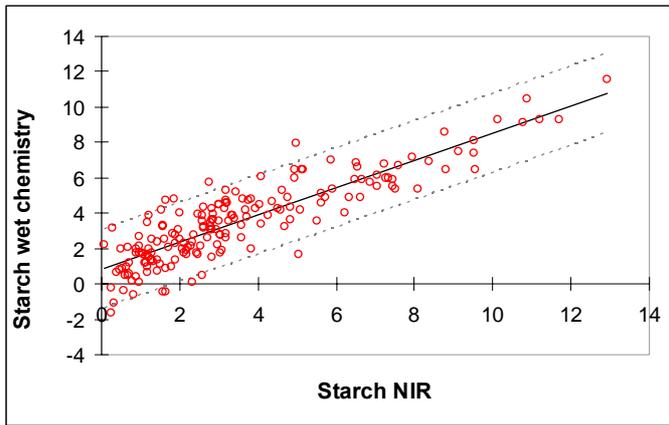


Figure 8- Correlations between predicted values (NIR) and reference values (laboratory or wet chemistry) for starch.

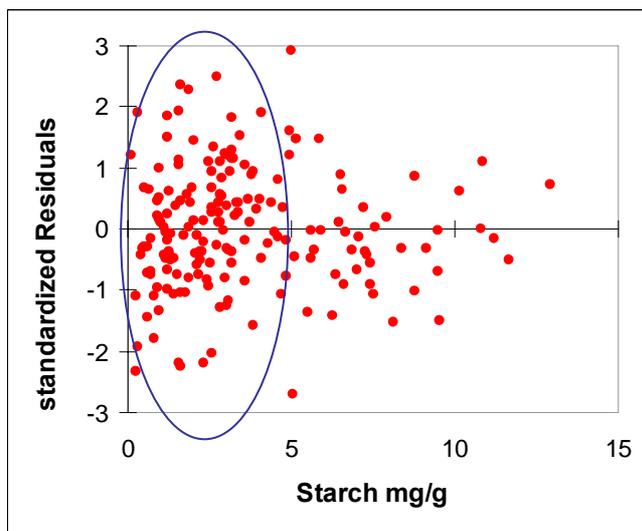


Figure 9- Distributions of the values of the reduced centred residues according to the starch concentrations.

On rachis

Descriptive statistic of reference values

The reference values of *rachis* concentrations were statistical described in table 4-. The distributions of glucose and fructose concentrations were dissymmetrical on the left: 65.6% of the fructose values were lower than 8.3 mg g⁻¹ and 64% of the glucose values than 7.8 mg g⁻¹. The distribution of the sucrose concentrations was normal. The distribution of the starch concentrations was dissymmetrical on the left with 55% of the values lower than 4.3 mg g⁻¹.

Table 4- Descriptive statistics of the reference concentrations (mg g⁻¹ DM) of rachis

| | glucose | fructose | sucrose | starch |
|-----------|------------|------------|-------------|------------|
| Values Nb | 295 | 295 | 295 | 115 |
| Range | 0.9 – 38.2 | 0.9 – 40.8 | 1.0 – 183.8 | 0.3 – 20.3 |
| Mean | 7.9 | 7.7 | 63.5 | 5.0 |
| SD | 6.6 | 6.5 | 29.6 | 4.2 |
| SEL | 5.3 | 6.6 | 5.6 | 7.9 |

NIRS Calibration and analysis of predicted values

The calibrations developed for the fructose, glucose and sucrose were powerful (R²= 0.94, 0.93 and 0.94; RPD > 3 for sucrose or close to 3 for fructose and glucose). The equation developed for the prediction of the starch concentrations was not very powerful (R² = 0.83 and RPD = 1.6).

Table 5- Statistical parameters of each sugar NIRS equations for rachis

| | N | moyenne | SD | SEC | R ² | SECV | CV (%) | RPD |
|----------|-----|---------|------|-----|----------------|------|--------|-----|
| glucose | 247 | 6.6 | 4.9 | 1.3 | 0.93 | 1.8 | 28 | 2.7 |
| fructose | 255 | 6.6 | 4.8 | 1.2 | 0.94 | 1.8 | 27 | 2.6 |
| sucrose | 250 | 61.5 | 27.1 | 6.7 | 0.94 | 8.0 | 13 | 3.4 |
| starch | 107 | 4.5 | 3.8 | 1.6 | 0.83 | 2.3 | 51 | 1.6 |

Figures 10 *for the whole samples* made it possible to judge visually quality of the adjustment for fructose, glucose, sucrose and starch and to locate the outliers. In this case, outliers are numerous: 35 in the case of glucose, 27 for the fructose, 32 for sucrose and 6 for the starch. These outliers were isolated for the calibration.

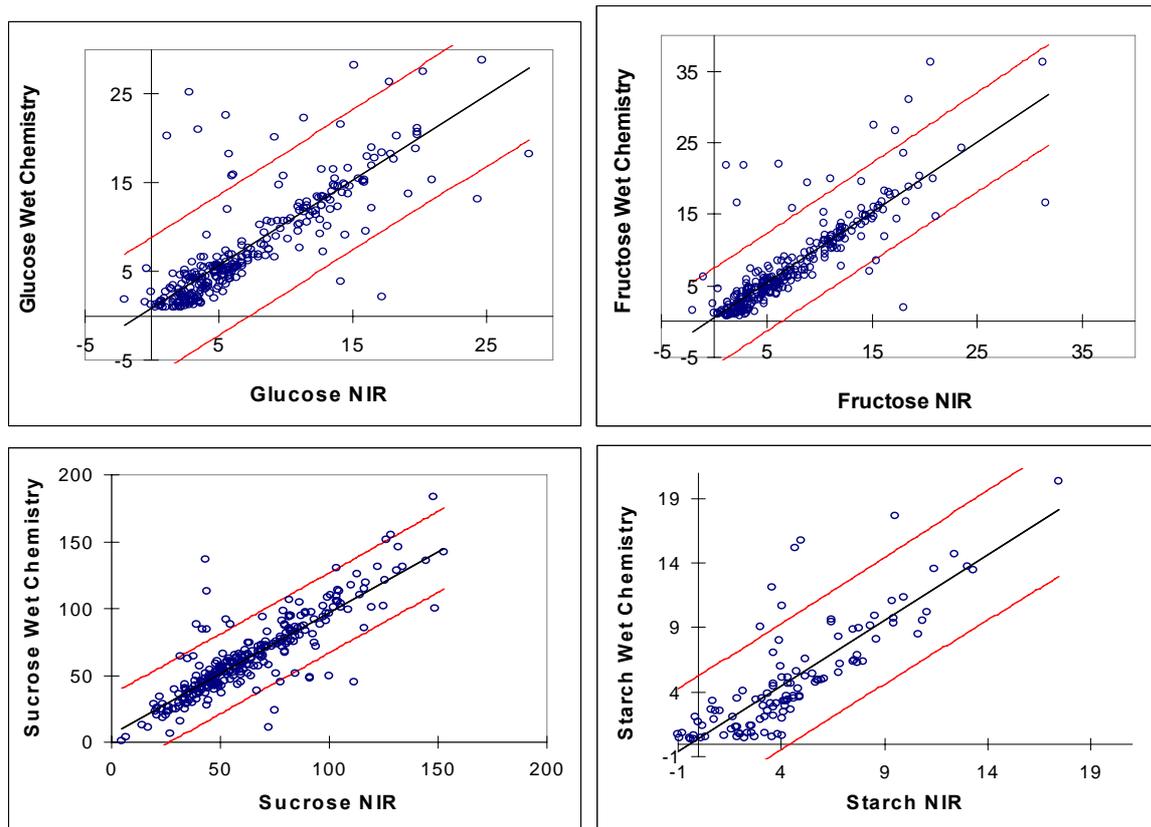


Figure 10- Correlations between predicted values (NIR) and reference values (laboratory or wet chemistry) for glucose (left, up), for fructose (right, up), for sucrose (left, down) and for starch (right, down).

On petioles

Descriptive statistic of reference values

The reference values of *rachis* concentrations were statistical described in table 6-. Distributions of fructose and glucose concentrations were dissymmetrical on the left, with extreme values higher than 50 mg g⁻¹ for the fructose and higher than 65 mg g⁻¹ for glucose. Distribution of sucrose concentrations followed a normal distribution. Distribution of starch concentrations was strongly dissymmetrical on the left: 61% of values were ranged between 0.2 mg g⁻¹ and 3.1 mg g⁻¹, 46% of values between 3.1 g g⁻¹ and 6.1 mg g⁻¹. SEL for starch was high; laboratory accuracy for this sugar in this compartment in particular was low. This observation can be explained by an internal reference whose starch concentration was naturally low.

Table 6- Descriptive statistics of the reference concentrations (mg g^{-1} DM) of petioles

| | glucose | fructose | sucrose | starch |
|-----------|------------|------------|-------------|------------|
| Values Nb | 425 | 425 | 423 | 236 |
| Range | 0.9 – 86.1 | 0.8 – 84.0 | 2.0 – 225.4 | 0.2 – 29.7 |
| Mean | 21.5 | 14.7 | 107.4 | 3.8 |
| SD | 15.6 | 13.8 | 44.1 | 4.5 |
| SEL | 5.3 | 5.9 | 5.6 | 21.7 |

NIRS Calibration and analysis of predicted values

On the basis of the Mahalanobis distances, seven samples were isolated to carry out the calibration. Calibrations developed for the fructose, glucose and sucrose were powerful ($R^2=0.98$, 0.97 and 0.94 ; RPD was clearly > 3). The equation developed for the prediction of starch concentration was powerful ($R^2=0.86$ and $RPD=2.0$), however less than equations developed for sugars (monosaccharides and sucrose). In this case, contrary to the leaflets, starch concentrations were continuous between 0.2 mg g^{-1} and 22.1 mg g^{-1} (figure 11).

Table 7- Statistical parameters of each sugar NIRS equations for petioles

| | N | Moyenne | SD | SEC | R^2 | SECV | CV (%) | RPD |
|----------|-----|---------|------|------|-------|------|--------|-----|
| glucose | 393 | 20.3 | 14.3 | 2.6 | 0.97 | 3.3 | 16 | 4.3 |
| fructose | 387 | 13.4 | 12.3 | 1.5 | 0.98 | 2.1 | 15 | 5.9 |
| sucrose | 388 | 105.2 | 43.3 | 10.2 | 0.94 | 11.9 | 11 | 3.7 |
| starch | 220 | 3.2 | 3.3 | 1.1 | 0.86 | 1.6 | 50 | 2.0 |

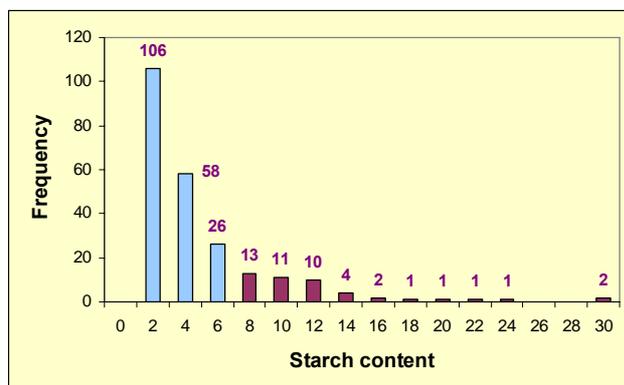


Figure 11 - Distribution of the starch concentrations in the petioles

Figure 12, *for only the samples used for the calibration*, made it possible to judge visually quality of the adjustment for fructose, glucose, sucrose and starch.

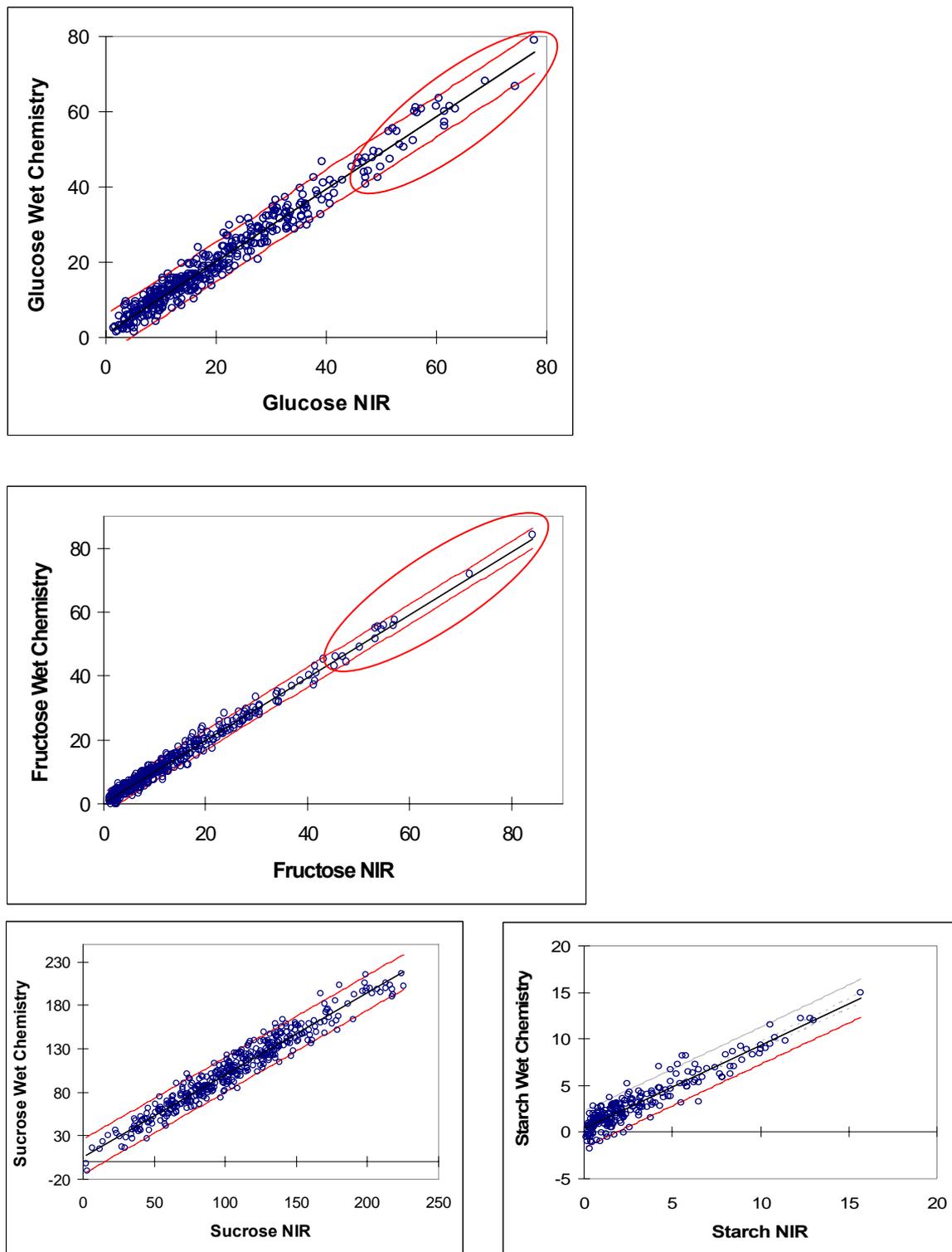


Figure 12- Correlations between predicted values (NIR) and reference values (laboratory or wet chemistry) for glucose (up), for fructose (middle), for sucrose (left, down) and for starch (right, down).

On the stem

Descriptive statistic of reference values

The reference values of *rachis* concentrations were statistical described in table 8-. Distributions of fructose and glucose concentrations were dissymmetrical on the left: for the fructose, 53% of the values were lower than 1.4 mg g⁻¹ and 54% of glucose concentrations were lower than 1.7 mg g⁻¹. Distribution of sucrose concentrations followed a normal distribution; two samples had concentration higher than 230 mg g⁻¹. Distribution of starch concentration was strongly dissymmetrical on the left

with 85% of values ranged between 0.03 mg g⁻¹ and 19 mg g⁻¹ and 67% of values lower than 4.7 mg g⁻¹.

Table 8- Descriptive statistics of the reference concentrations (mg g⁻¹ DM) of stem

| | glucose | fructose | sucrose | starch |
|-----------|------------|------------|-------------|--------------|
| Values Nb | 167 | 167 | 167 | 164 |
| Range | 0.2 – 16.2 | 0.1 – 13.2 | 4.8 – 257.1 | 0.03 – 188.8 |
| Mean | 2.7 | 2.1 | 98.7 | 11.2 |
| SD | 3.0 | 2.4 | 39.5 | 26.0 |
| SEL | 4.7 | 7.0 | 5.0 | 36.1 |

NIRS Calibration and analysis of predicted values

In the case of sucrose, adjustment of concentrations was passable (R²= 0.92 and RPD = 2.5), 7 outliers were eliminated. However SECV (= 13.7) is definitely higher than SEC (= 9.9). So the model robustness and stability for sucrose were insufficient, in other words the model was perfectible in prediction. Equations of glucose, fructose and starch are not exploitable, because concentrations distributions were dissymmetrical and concentrations low. Absorbance was too weak and not variable enough. For starch in particular, results illustrated well the difficulty to adjust data whose distribution is dissymmetrical and more of 50% of values were lower than 1 mg g⁻¹.

Table 9- Statistical parameters of each sugar NIRS equations for stem

| | N | mean | SD | SEC | R ² | SECV | CV (%) | RPD |
|----------|-----|------|------|-----|----------------|------|--------|-----|
| sucrose | 154 | 95.5 | 34.6 | 9.9 | 0.92 | 13.7 | 14 | 2.5 |
| starch | 150 | 6.4 | 13.1 | 3.2 | 0.94 | 5.7 | 13 | 2.3 |
| glucose | 147 | 1.8 | 1.5 | 1.1 | 0.45 | 1.3 | 70 | 1.2 |
| fructose | 146 | 1.3 | 1.1 | 0.8 | 0.37 | 1.0 | 72 | 1.1 |

To improve the starch model, two starch concentration classes were created on the basis of laboratory reference values: (i) the first one with concentrations lower than 10 mg g⁻¹, (ii) the second one with concentrations higher than 10 mg g⁻¹. A linear factorial discriminating analysis was carried out by taking the first 10 components extracted from the PCA realized for the whole spectra for stem, like explanatory variables. The discriminating model obtained classed correctly 88.4% of the observations. 95.3% of the concentrations, lower than 10 mg g⁻¹, were well classified; only 6 samples were badly classified. 65% of the concentrations, higher than 10 mg g⁻¹, were well classified; 13 samples were badly classified, 9 had starch concentrations ranged between 10 mg g⁻¹ and 21 mg g⁻¹, two had concentration of 33 mg g⁻¹ and 2 had concentrations ranged between 57 mg g⁻¹ and 59 mg g⁻¹. In cross validation, 50 samples were drawn at random; 37 were lower than 10 mg g⁻¹ and 13 higher than 10 mg g⁻¹. The rate of correct classification was 86%.

On coconut kernel

See the communication which follows, entitled "Physico-Chemical changes in the fruits of two coconut (*Cocos nucifera* L.) hybrids during ripening. A NIRS-boosted study" (Prades *et al.*, 2005).

On Mango tree

Population Structure

An ACP realised on all spectra made it possible to highlight the groups corresponding to the various vegetative and fruit compartments (figure 13). In particular roots, stump and taproot were well differentiated according to the axe 1.

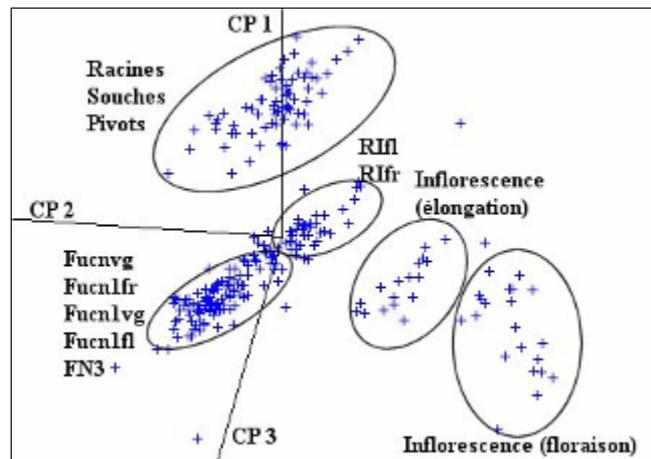


Figure 13- Distribution of the mango tree spectra according to the first 3 CPs (91.9% of native variance).

Descriptive statistic of reference values for roots

83 root samples were analysed in the laboratory for their concentrations of inositol, sucrose, glucose, fructose and starch. These 83 samples were composed of 66 fine, medium, large roots near of the taproot and to 2m from it, 9 taproots and 8 stumps. The reference values of *root* concentrations were statistical described in table 10.

Table 10- Descriptive statistics of the reference concentrations ($mg\ g^{-1}\ DM$) of roots

| | inositol | glucose | fructose | sucrose | starch |
|-----------|-----------|-----------|-----------|------------|--------------|
| Values Nb | 73 | 73 | 73 | 73 | 73 |
| Range | 0.8 – 2.4 | 0.2 – 6.6 | 0.1 – 6.7 | 9.2 – 33.7 | 34.1 – 387.0 |
| Mean | 1.5 | 1.6 | 1.8 | 19.2 | 211.4 |
| SD | 0.4 | 1.3 | 1.4 | 5.7 | 90.4 |

NIRS Calibration and analysis of predicted values

The statistical parameters of the various developed equations are gathered in table 4. The equations are developed for each criterion for the whole of the samples (roots, stumps and taproots).

Table 11- Statistical parameters of each sugar NIRS equations for roots

| Component | N | Mean | SD | SEC | R ² | CV (%) | SECV | RPD |
|-----------|----|-------|------|------|----------------|--------|------|-----|
| Inositol | 72 | 1.5 | 0.4 | 0.2 | 0.71 | 17 | 0.3 | 1.6 |
| Glucose | 69 | 1.4 | 0.9 | 0.6 | 0.56 | 46 | 0.7 | 1.4 |
| Fructose | 71 | 1.7 | 1.3 | 0.5 | 0.82 | 40 | 0.7 | 1.8 |
| Sucrose | 71 | 18.8 | 5.2 | 3.0 | 0.68 | 19 | 3.7 | 1.4 |
| Starch | 69 | 210.3 | 92.6 | 16.6 | 0.97 | 9 | 21.0 | 4.4 |

The equations developed for inositol, glucose, fructose and sucrose were not very powerful ($R^2 < 0.85$, $RPD < 2$). The performances of the equation developed to predict the starch concentration were remarkable ($RPD = 4.4$, $R^2 = 0.97$). Figure 14, *for only the samples used for the calibration*, made it possible to judge visually the quality of the adjustment for starch.

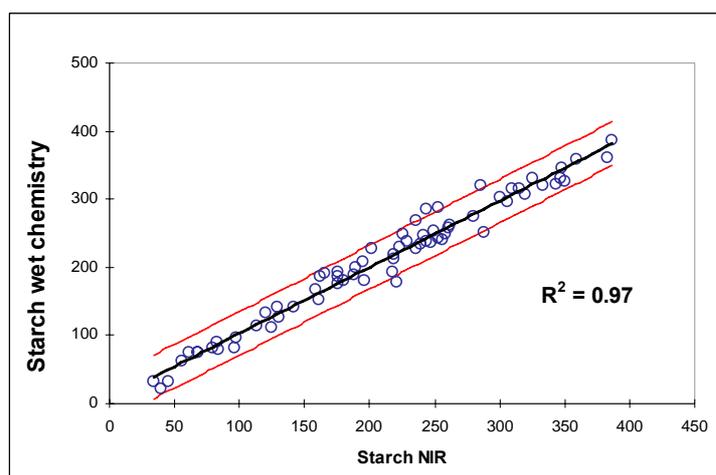


Figure 14- Correlations between predicted values (NIR) and reference values (laboratory or wet chemistry) for starch.

NIR Value Accuracy: synthesis

In seven cases listed in table 12, the developed models made it possible to classify the samples into two or three distinctive groups:

- For the leaflets, for the determination of the starch in the three compartments of the fronds and the stem;
- For the determination of sucrose in the stem;
- For the determination of the lipids in the coconut kernel with three groups centred on 30, 40 and 50%.

In five cases, no robust determination can be made. It acted:

- Determination of monosaccharides in the stem;
- Determination of soluble sugars in the mango roots.

For the remaining determinations, the average values obtained and the dispersion of these values remained in all the cases comparable with those obtained on the reference data.

Table 12- Mean concentrations (mg g^{-1} or % for kernel) \pm SD of sugars and starch estimated by NIR models for coconut and mango.

| | | glucose | fructose | sucrose | starch | TSS | Lipids |
|---------|----------|--|----------------|----------------------|--|---------------|----------|
| COCONUT | leaflets | 9.6 ± 3.9 | 10.0 ± 3.3 | 23.0 ± 8.0 | 2 groups ($>$ and $<$ 4 mg g^{-1}) | - | - |
| | rachis | 2 groups ($>$ and $<$ 10 mg g^{-1}) | | 61.0 ± 16.0 | 2 groups ($>$ and $<$ 4 mg g^{-1}) | - | - |
| | petiole | 13.0 ± 4.0 | 21.0 ± 6.0 | 105.0 ± 24.0 | 2 groups ($>$ and $<$ 4 mg g^{-1}) | - | - |
| | stem | - | - | 2 groups (to define) | 2 groups ($>$ and $<$ 10 mg g^{-1}) | - | - |
| | kernel | | | | | 9.0 ± 1.6 | 3 groups |
| MANGO | root | - | - | - | 210.0 ± 42.0 | - | - |

Conclusions & Perspectives

For *coconut stem and fronds*, the calibrations developed for soluble sugars (monosaccharides and sucrose) for the three fronds compartments described (leaflets, rachis and petiole) could be applied in routine for coming determinations. In these cases, the R^2 observed ($>$ 0.8) showed that relations between NIR predicted values and sugar reference values were significant. On the other hand, the models developed for the starch were not enough powerful and robust to be used on these three

fronds compartments. Currently, the calibrations developed on the stems were not exploitable for monosaccharides, unstable and not very powerful for sucrose and starch. The NIR good results or, *a contrario*, the bad results depended on sugar and starch concentrations values; the NIR sensitivity failed for low concentrations ($< 10 \text{ mg g}^{-1}$).

For **Mango tree**, the calibrations developed for starch on root system could be applied in routine for determinations to come. Actually, this was not the case of soluble sugars on this compartment. The developments of calibrations for all other compartments are in progress.

Why? The main reasons of this report (in particular, on coconut) were due to

- The dissymmetry observed in the distributions of the different compounds. The highest frequency in distribution was observed for very low concentrations values;
- The limit of NIR detection (around 10 mg g^{-1}) was sometimes reached (in particular for the low concentrations);
- The inaccuracy (especially for low content) of reference values.

Was NIR calibration accuracy close to wet chemistry accuracy? The NIR error (CV) was almost twice higher, but in some cases (glucose, fructose and sucrose in leaflets; sucrose in rachis; TSS in kernels and starch in mango tree roots) this error was quite close to the average laboratory error (as the lab error observed was depending on the content).

Was it possible to make same conclusions or closed conclusions with classical and NIRS methods? The answer was "yes" on coconut, for the two described vegetative compartments and for valid NIR models. However, this point must be still gone into more deeply by rigorously comparison with the conclusions made from the laboratory data (Mialet-Serra, 2005; Mialet-Serra *et al.*, 2005). In particular if the dispersion of the values is the same or of the same order of magnitude in the both cases, it will be then possible to lead to the same conclusions in term of reserves pool quantity estimated but also of variations of these quantities.

NIR models were perfectible. In order to improve the models, we could

- Add new values to equilibrate distributions and work with subgroups;
- Test new regression methods like PLS2 (well adapted to sugar values) or neuronal network;
- Improve (modify) analytical protocol between NIR and wet chemistry;
- Create artificial samples (mixture) to cover the whole range and create single group (e.g. one group for fronds) could represent practical solutions which will contribute to consolidate these first models.

For **coconut kernel**

See the conclusions in the communication which follows, entitled "*Physico-Chemical changes in the fruits of two coconut (*Cocos nucifera* L.) hybrids during ripening. A NIRS-boosted study*" (Prades *et al.*, 2005).

References

- Davrieux, F., 1997 – Application de la spectrométrie proche infrarouge à la caractérisation de mélanges de cafés torréfiés moulus. Mémoire DESS : Méthodes statistiques appliquées aux industries agronomiques, agro-alimentaires et pharmaceutiques. Université de Montpellier 2. 91 p.
- Lachenal, G., 1998 – Méthodes d'analyse rapide par spectroscopie dans l'infrarouge proche et moyen. Bertrand, D. Ed., Lavoisier. Paris.

- Mialet-Serra, I., 2005 – Rôle et gestion des réserves carbonées face à la variabilité du climat chez une monocotylédone arborescente, le cocotier (*Cocos nucifera* L.) : Analyse et bilan. Th. : Ecophysiologie : ENSA de Montpellier, 112 p. + 6 annexes.
- Mialet-Serra I., Clément A., Sonderegger N., Roupsard O., Jourdan C., Labouisse J-P. & Dingkuhn M., 2005 - Assimilate storage in vegetative organs of coconut (*Cocos nucifera* L.). *Experimental Agriculture*, 41 (2): 161-174.
- Normand F., Lagier S., Escoute J., Verdeil J-L., Mialet-Serra I., 2006 - Starch localisation in mango tree: histological observations. Communication présentée au 8th *International Symposium on Mango*, 5-10/02/2006, Sun City, South Africa. In press in *Acta Horticulturae*.
- Thuriès, L., Bastianelli, D., Davrieux, F., Bonnal, L., Olivier, R., Pansu, M., Feller, C., 2005 – Prediction by near infrared spectroscopy of the composition of plant raw materials from the organic fertiliser industry and of crop residues from tropical agrosystems. *J. Near Infrared Spectrosc.* 13, 187-199.



Cf. Annexe

BIOCHEMICAL INDICATORS FOR CARBON METABOLISM

By Agnès GUILLIOT, Anne CLEMENT-VIDAL, Pisamaï CHANTUMA, Sandrine LEGROS.

UMR 547, P.I.A.F, Université Blaise Pascal, Clermont-Ferrand, France; CIRAD, RU 59 "Integrative modeling", Montpellier, France; RRIT, Thaïlande ; CIRAD, RU 34, « PerSyst : Performance of tree crop-based systems », France/Indonesia.

Introduction

In the source to sink relation, limiting enzymes can be regarded as biochemical indicators of source or sink strength, as they regulate the cell metabolism. In fact, enzyme activity may be affected by both external (biotic and abiotic stress) and internal conditions and the quality and the amount of carbohydrates formed depend upon the enzymes present and their regulation. It is now well recognized that enzymes play important roles in carbohydrates partitioning.

Key enzymes determination allows a better understanding of carbohydrate metabolism.

Moreover, key enzymes determination makes it possible to find some indicators of a mechanism (storage, mobilisation, exportation ...) and of a physiological character (yield, stress resistance ...).

The finding of biochemical indicators for carbon metabolism implies:

- A careful exhaustive biochemical bibliography;
- The determination of enzyme activities to find reactions which are catalysed in the studied material;
- The correlation of the measured enzyme activities with known carbon flux to estimate if the activity could account for the process taking place (concept of limiting enzyme).

Enzymatic determinant of sink strength: some examples

Potato tuber (Solanum tuberosum) example

As the translocated sucrose is the starting point of the tuber's metabolism, the manner in which it is partitioned between respiration, biosynthesis and storage is central to the growth and metabolism of the tuber. Knowledge of the control of the sugar content is important in respect of both the economy and the economic use of the tuber. For these reasons,

Morrell and ap Rees (1986) studied sugar metabolism in developing tubers. The author's question was about the role of invertases (INV) and sucrose synthase (Susy) in the sucrose metabolism of potato tuber (*Solanum tuberosum*)? In fact, after sucrose unloading out of the phloem, sucrose can be broken down by cell wall INV (cwINV) and then enter the cell as hexoses, or enter the cell as sucrose and be metabolized by Susy or cytosolic INV (cINV). Their measurements of catalytic activities of sugar metabolism enzymes, on three varieties of potato, suggest that much of the sucrose translocated to the developing tuber was metabolized via sucrose synthase.

To investigate the unique role of sucrose synthase with respect to sucrose metabolism and sink strength of growing potato tubers, an antisense strategy was set up by Zrenner *et al.* (1995). Transgenic potato plants were created expressing Susy antisense RNA. The inhibition of Susy leads to no change in sucrose tuber content but to a strong accumulation of reducing sugars and an inhibition

of starch accumulation in developing potato tubers. The changes in carbohydrate accumulation are accompanied by a decrease in total tuber dry weight. Altogether these data were in agreement with the assumption that sucrose synthase was the major determining enzyme of potato tuber sink strength.

Tomato fruit (Solanum lycopersicum) example

In breeding for tomato fruit quality, there is a strong focus on the capacity of fruit cells to accumulate and store carbohydrate. Tomato fruits have 3 phases of development: during the first phase of fruit development (0-10 days after anthesis) the growth is slow and starch accumulate; then (10-40 days after anthesis), a rapid growth begins and there is a rapid hexose accumulation and apoplastic transport. ; the third phase consist of fruit maturation.

A positive correlation was demonstrated between starch accumulation and tomato yield. Moreover, in earlier sink strength work with tomato fruits, workers concluded that "sink activity" was a more primary determinant of sink strength than sink size. From these results, the question arose on the enzymatic determinant for starch storage during phase 1 of tomato fruit development. Sun et al. (1992) showed a positive correlation between tomato fruit size (or mass) and Susy activity, illustrating the contribution of Susy to tomato fruit sink strength. Therefore, sucrose synthase could serve as an indicator of sink strength in growing tomato fruits. Other studies (Wang *et al.* 1993) investigated the relative roles of sucrose synthase and acid invertase in the determination of sink strength in tomato plants. Susy activities did not correlate with starch accumulation during the first 7 days of fruit development suggesting that sucrose synthase is not the only critical enzyme in starch. In fact, Schaffer *et al.* (2000) compared two breeding line of tomato, characterized by a different transient starch accumulation in the young fruit. They showed that the increase in starch accumulation was correlated with an increase in ADPglucose pyrophosphorylase (AGPase) activity. This suggests that native tomato AGPase activity is limiting to sucrose-to-starch flux; furthermore, enzyme activity and flux can be increased utilizing natural genetic variability for a regulated plant ADPglucose pyrophosphorylase. These works have shown that Susy and AGPase contribute and are determining to sink strength of tomato fruit during phase 1.

As fruit development progress, starch accumulation slows and hexoses accumulate. During the second phase of tomato fruit development cwINV activity is high suggesting an importation of sugar as hexoses. Ruan *et al.* work (1997) reports an assessment of whether variation in the capacity for hexose retrieval from the fruit apoplast contributes to the regulation of sugar accumulation during phase 2. The approach compared two genotypes, a high hexose line and a low hexose line, which differ in fruit hexose content. The results support the hypothesis that the activity of energized hexose carriers on the plasma membranes of storage parenchyma cells is a significant determinant of the genotypic difference in hexose accumulation.

Conclusion

Biochemical investigations during the last years have pointed to a few enzymes which, due to their key biochemical role, were considered possible contributors to sink or source strength. A schematic drawing of carbohydrate metabolism of source and sink organ and the biochemical determinants of source and sink strength is presented below (Figure 1).

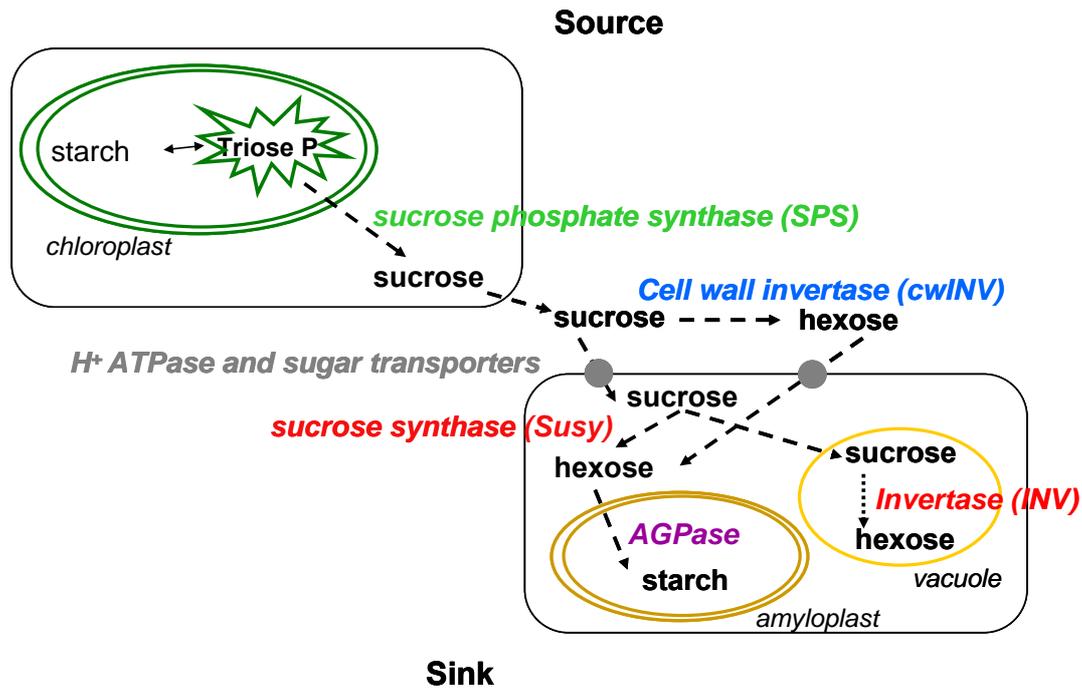


Figure 1- Schematic drawing of enzymes of carbohydrate metabolism which are contributors to sink or source strength. The sucrose is either taken up into the cell, or hydrolyzed by cell wall invertase and taken up into the cell as hexoses. H⁺-ATPase and sugar transporter are involved in the apoplastic transport of sugars. Sucrose in the cytosol may either be taken up into the vacuole, be hydrolyzed by cytosolic invertase to corresponding hexoses or be cleaved by sucrose synthase. Sucrose in the vacuole can be hydrolyzed by soluble acidic invertase. Starch is synthesized in the amyloplast. AGPase : ADP glucose pyrophosphorylase.

Assessment on enzymatic studies on two “ATP reserves” species: the rubber and the oil palm trees

Two plant programs planned to study sugar metabolism through activity of different enzymes: hevea and oil palm tree. Unfortunately these aspects of the project were developed recently, the preliminary knowledge of carbohydrates partitioning in the plant was necessary to start this topics.

On hevea- Thus hevea started to adapt methods used on woody samples in walnut and peach tree into rubber trunk samples (bark and wood). The hypothesis tested is whether or not these enzymatic activities could related to latex production, phenology and could be used to understand the mechanisms of carbon partitioning involved in the different tapping system. In addition it was shown that the latex production is linked to intralaticiferous sucrose content then certainly with the enzymes responsible of its synthesis and transport around latex vessels (parenchyma). Now three enzymatic methods were adapted: total amylases commonly involved in starch reserves mobilisation, sucrose-phosphate synthase (SPS) to estimate the sucrose export, cell wall invertase (CWI) involved in the sucrose transport in cell in hexose form linked to source/sink relationships. Moreover starch is the more variable sugar in the trunk of tapping tree compared to control tree and we suggest to measure also the sucrose synthetase activity (SuSy) associated to starch synthesis. Indeed it seems that the latex production involves an increase of starch content in the trunk. Thus the next step will consist to compare double cut alternative (DCA) tapping system with classic tapping system (D2) (see description in hevea part by P. Thaler *et al.*) especially when the production increase strongly (October) through the analysis of these enzymatic activities.

On oil palm- “ATP reserves” project on oil palm tree, made it possible a determination and a quantification of non structural carbohydrates (NSC: glucose, fructose, sucrose, starch) in the various organs of the plant. This approach took into account at the same time the plant phenology and the

effects of various treatments (fruit pruning, leave pruning) on the distribution of these glucids (see part oil palm tree by I. Mialet-Serra *et al.*). The preliminary results made it possible to firstly locate the NSG in the stem.

In this part a finer analysis of the NSC localization was carried out (Figure 2). These first results highlight an increasing content of NSC from the stem stump to the stem top. Among these NSC, glucose is most strongly represented between the stump and the medium of the stem then it decrease. The starch seems to form a pocket towards the top/sub apical area of the stem, whereas it is represented little on the basal area. Finally the sucrose whose content is relatively stable until the top of the stem (weak positive gradient) increases very strongly in the meristematic area. The fructose contrary to glucose is very slightly represented throughout the stem.

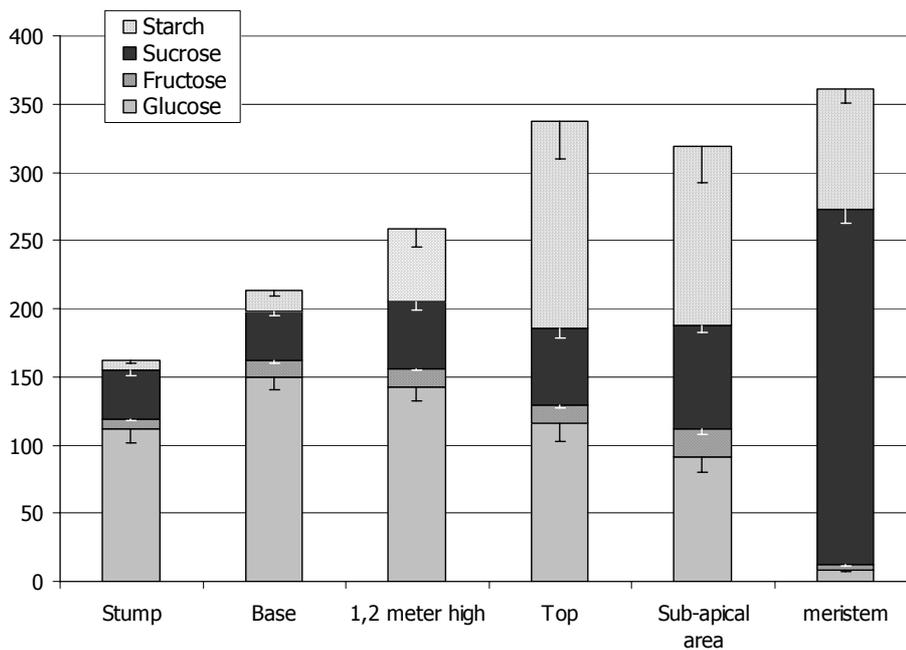


Figure 2- Glucose, fructose, sucrose and starch distribution along the stem of oil palm tree (on control trees and without seasonal aspect).

Then meristematic area appeared interesting to understand mechanisms involved in source/sink relationships, point out enzymes and to adapt enzymatic analysis methods. The targeted enzymes are acid invertases (vacuolar and cell wall), sucrose synthetase, sucrose phosphate synthase and amylase. The sampling will be carried out in this area according to the following diagram (Figure 3).

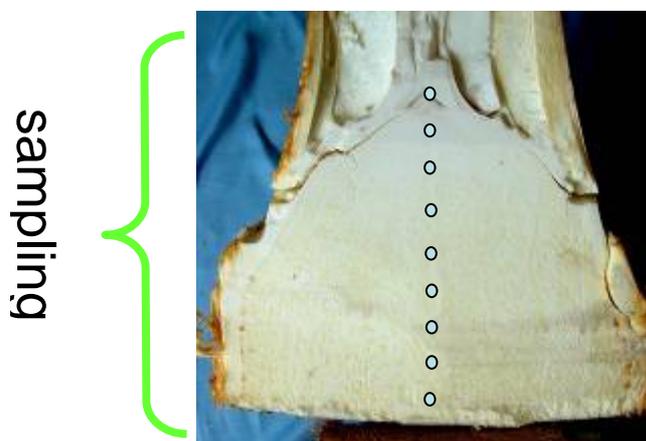


Figure 3- Longitudinal section of the top of the stem (sub-apical area), and localisation of the 9 samples used.

We hope that these studies will help us to answer various questions as: Are there efficiency for carbon management according to the genotype of plant and are there an adaptive response to stress? The ultimate goal would be to define markers used as tool for breeding.

References

Morrell and ap Rees, 1986- *Phytochem.* 25:1579-1585

Ruan *et al.*, 1997 - *Plant, Cell Env.* 20 :341-349

Schaffer *et al.*, 2000- *Plant Science* 152 :135–144

Sun *et al.*, 1992- *Plant Physiol.* 98:1163-1169

Wang *et al.*, 1993- *Plant Physiol.* 101:321-327

Zrenner *et al.*, 1995- *Plant J.* 7:97-107



Part 2- Towards an integrated approach...

**ROLE OF CARBON RESERVES IN PALM FAMILY CROPS:
AN EMERGING MODEL AND CONSEQUENCES FOR RESEARCH STRATEGY**

By Michael DINGKUHN, Isabelle MIALET-SERRA, Sandrine LEGROS, Anne CLEMENT-VIDAL
Nicole SONDEREGGER and Jean-Claude COMBRES.

RU 59 "Integrative Modeling", CIRAD, AMIS Department, Montpellier, France.

Problem statement

The ATP project's initial hypothesis on the function of transitory carbon reserves in perennial crops was that of a physiological buffer. Such a buffer would provide a supplemental source of assimilates during times of shortage (when demand is high due to fruit load, or when supply is low due to low radiation or stress). Conversely, during times of low demand, the plant would store excess assimilates in the form of sugar reserves. In fact, fruit production in oil palm is highly seasonal and tends to follow genotype and site specific rhythms (Figure 1: case of Deli x Lamé genotype at Pobé, Indonesia).

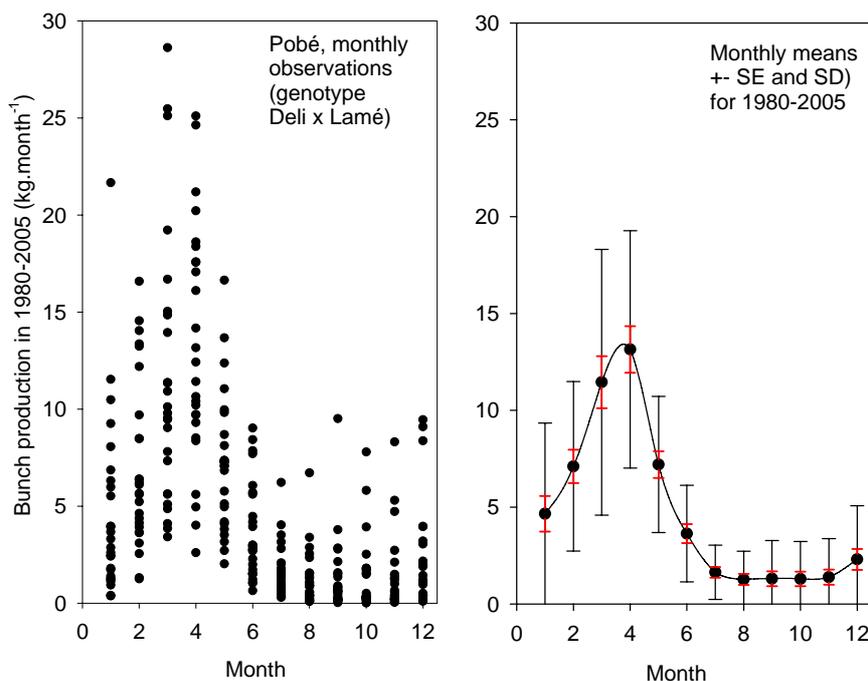


Figure 1- Seasonal rhythm of bunch biomass production of oil palm at Pobé.

This seasonality of production cannot be explained with climatic factors alone, such as drought, temperature or light resources. It is bound to cause temporary, sink-source imbalances, which might in turn necessitate transitory carbon reserves as stabilising factor. We will in the following summarize our current understanding of these processes, formalised in the form of a model, and then discuss the consequences of our theories for future research on oil palm improvement.

Causes of seasonal rhythms of production

The current "best bet" hypothesis on seasonal peaks of flowering and fruiting is photo-periodism, which is the effect of day length on floral induction. We suggest that this mechanism operates at the

phytomer scale, with short days accelerating flowering. Thus, under long days phytomers “queue up” and later flower in rapid succession (Figure 2).

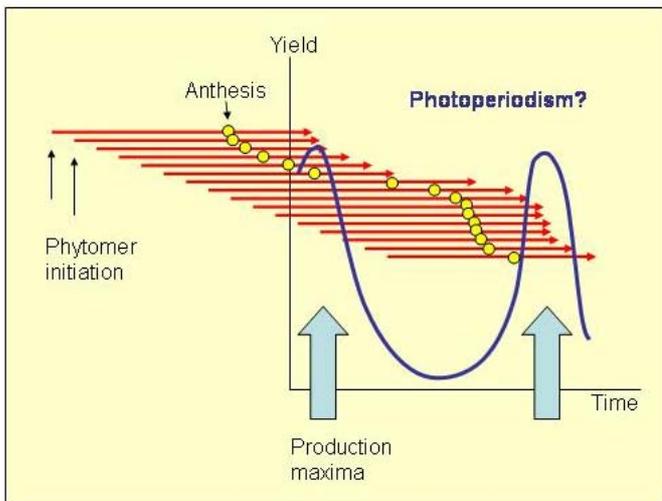


Figure 2- Schematic diagram explaining seasonal rhythms of oil palm flowering with photoperiodism at the phytomer scale.

Theory of buffer function for reserves

Following previous observations on coconut (Mialet-Serra, 2005; Mialet-Serra *et al.*, 2005), we propose that vegetative development and growth (leaf area, trunk & root) of oil palm is much more constant, or less plastic, than reproductive processes (Legros *et al.*, 2006; Siregar, 2006 (in press)). Supposing that canopy photosynthesis is a direct function of leaf area index and climate, and therefore potentially quite stable in the humid tropics, the seasonal rhythms of fruiting would thus cause sink-source imbalances requiring compensation by stored assimilate reserves (Figure 3).

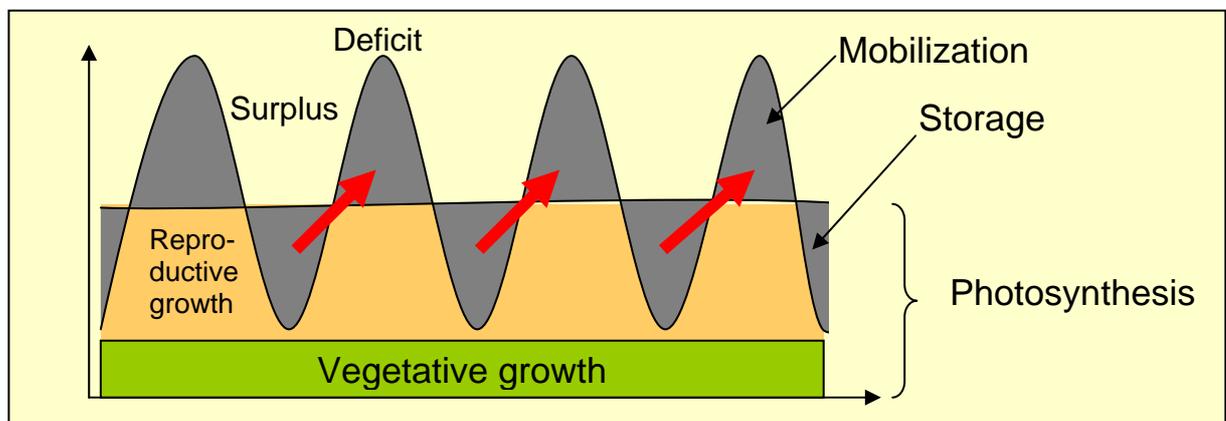


Figure 3- Hypothetical, compensatory function of carbon reserves.

The Ecopalm model

The Ecopalm model in its current version (V.3) is based on the hypotheses described above, as well as the concept of an internal index of competition ($I_c = \text{assimilate supply/demand}$), which links assimilate abundance to development processes (Combres *et al.*, 2003). The model captures well the seasonal and inter-annual fluctuations of production (Figure 4: fresh weight of bunches). It also simulates fairly accurately bunch and fruit number, although full validation will only be

possible once suitable data sets are available. The model does simulate transitory reserve storage and mobilization, but only on a purely theoretical basis because such data were so far unavailable.

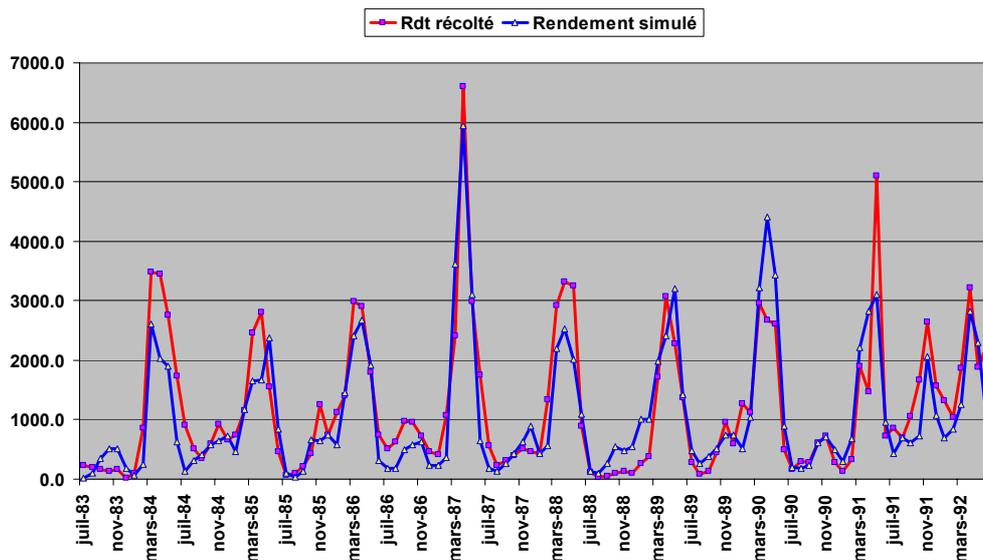


Figure 4- Observed and simulated, monthly fruit fresh weight production (kg.ha^{-1}) for 1983-91 in Ivory Coast.

Observed carbon reserve dynamics in oil palm

The ATP project recently produced the first data on reserve dynamics in oil palm (Figure 5). They show strong seasonal, rhythmic variation in storage, similar to those observed in fruit production. Furthermore, reserve levels were much higher in oil palm trees whose inflorescences were systematically removed before fruit set, as compared to controls.

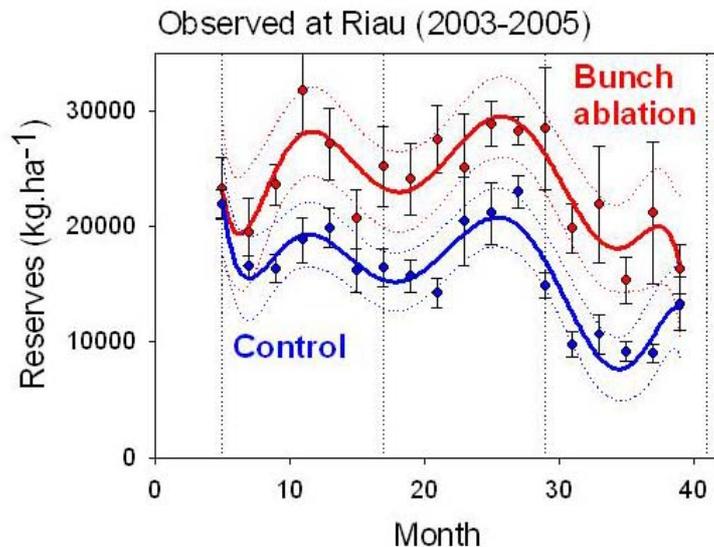


Figure 5- Dynamics of total sugar (starch and soluble sugars) in vegetative organs of oil palm, with and without removal of inflorescences, in the course of 3 years.

Consequently, the hypothesis that reserve levels respond to assimilate supply/demand imbalances is true. However, absolute levels and the seasonal amplitude of their oscillations were only half of the values simulated (Figure 6). Although the simulated and observed data are not fully comparable (the data sets currently available for Riau are insufficient for model implementation), it seems clear that the model over-estimates absolute reserve levels and the amplitude of their oscillations.

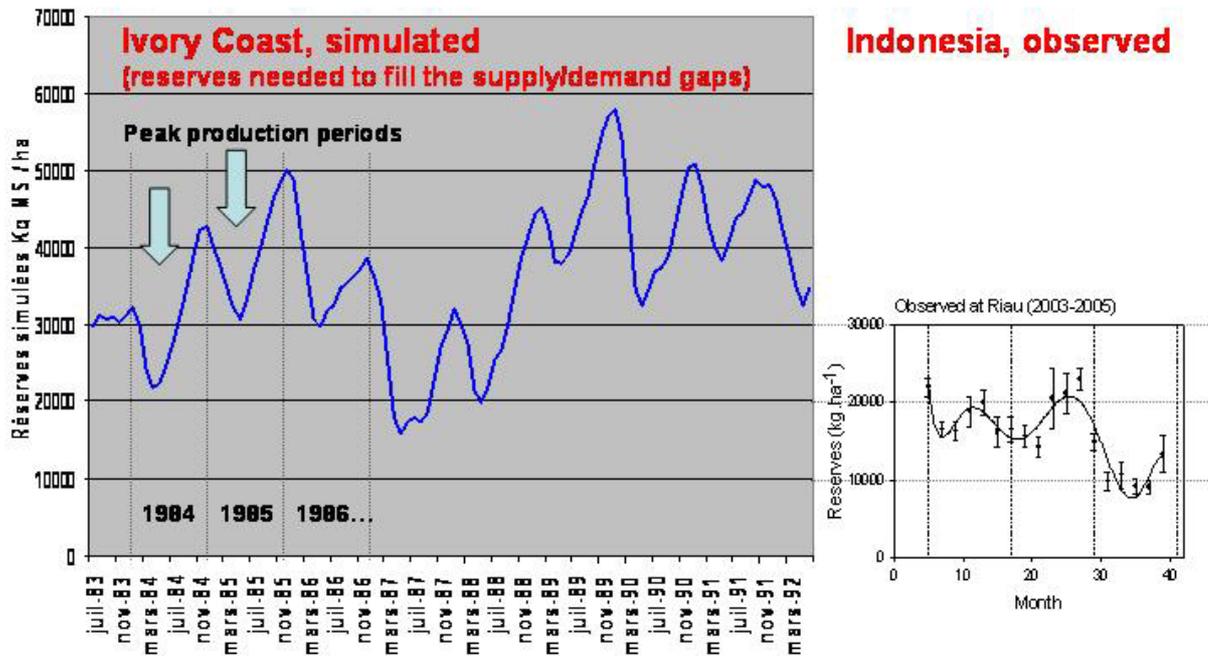


Figure 6- Simulated oil palm reserve fluctuations in Ivory Coast (left) and observed values in Indonesia (right, from Figure 5, adjusted to same scale). The two results are not fully comparable (different sites, genotypes, years) but suggest that the model over-estimates reserve levels.

A possible explanation of model errors: feed back inhibition of photosynthesis?

If reserve fluctuations are indeed smaller than one would expect from production kinetics and estimated carbon assimilation, the plant must dispose of additional adjustment mechanisms that explain the discrepancy. A possible way of tracking such mechanisms is the establishment of a carbon balance from a detailed growth analysis. On the basis of this carbon balance, which include maintenance (R_m) and growth respiration (R_g), the gross photosynthetic rates (P_{gross}) of the plant population can be estimated. From this, the gross radiation use efficiency can be estimated from equation (1):

$$RUE_{gross} = P_{gross} / PAR_{intercepted} \quad (\text{Equation 1})$$

with RUE = radiation use efficiency, P = photosynthesis, PAR = photosynthetically active radiation

The EcoPalm model assumes that RUE_{gross} is constant under drought-free conditions, which means that P_{gross} is a direct function of PAR intercepted by the leaf canopy. For coconut, we recently found that this is inaccurate (Figure 7), and it seems likely that this is also the case for oil palm. Total pruning of the inflorescences decreased RUE of coconut by 50%, whereas partial leaf pruning increased it slightly. Consequently, the plant seems to reduce its photosynthetic rate under conditions of low demand for assimilates. The underlying mechanism might be end product inhibition of accumulated sugars on carbon assimilation. This phenomenon might explain why oil palm and coconut accumulate less reserves than predicted under conditions of low demand (low reproductive sink capacity). We are currently verifying this hypothesis by direct measurements of leaf photosynthesis. If true, the EcoPalm model needs to be adjusted to take this response into account.

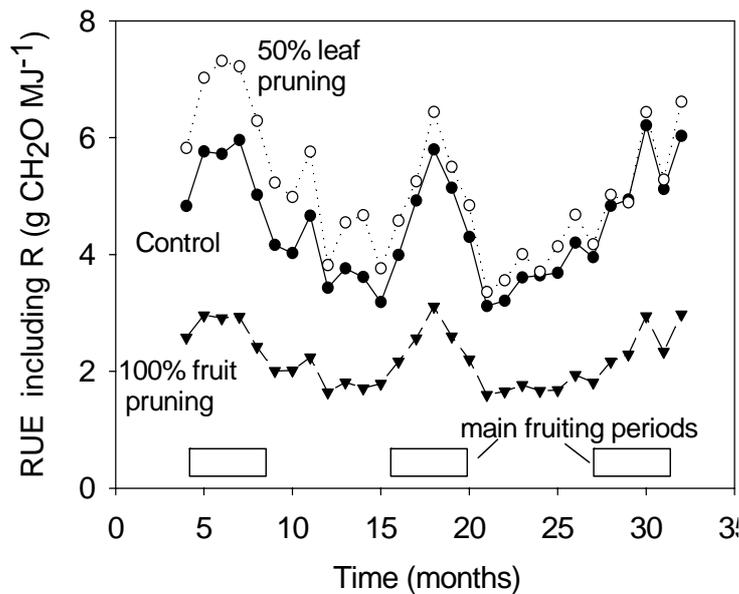


Figure 7- Dynamics of gross radiation use efficiency (RUE) of coconut trees under control conditions and leaf or fruit pruning in Vanuatu (Source: Mialet-Serra et al. (in preparation)).

- Adjustment of hypotheses
- Towards a coherent theory
- Consequences for applied research

Conclusion: Consequences for palms improvement strategies

The results presented here, once validated, provide an entirely new theoretical concept for the analysis of oil palm and coconut eco-physiology and agro-ecology, based on the following:

- Seasonal rhythms of fruiting are apparently based on **photoperiod sensitivity**. This mechanism has a relatively simple genetic basis and thus can probably be modified through breeding or transformation. Studies on the same genotypes at different latitudes are needed to confirm this.
- Sexualisation (case of oil palm), abortion probability and bunch size are affected by competition for assimilates among sinks, constituting an important **feedback of assimilate status on phenology**.
- Large amounts of (mainly soluble) **sugars constitute a buffer reservoir for sink/source imbalances**. Their role under stress conditions needs further study.
- Coconut, and probably also oil palm, have **extremely variable RUE**, indicating that **photosynthetic rates are sub-maximal during much of the year**. They might thus be amenable to genetic improvement, for example by reducing the seasonal oscillations of fruiting.

All of these conclusions are preliminary and need to be validated. Furthermore, the EcoPalm model needs to be updated, particularly with respect to conclusion 4, in order to accurately represent the main mechanisms through which the plant adjusts growth and production to variable environments. Lastly, there is a need to study the behaviour of different genotypes in order to understand the genetic diversity for the physiological traits described here. The Ecopalm model can thereby be instrumental in characterizing this genetic diversity.

Trait characterization through model parameterization (model assisted phenotyping) is a new approach in crop improvement (Dingkuhn, 2006). It requires further methodological work, particularly

the development of economical "minimum data sets" that can be measured on a large number of plants, and efficient model parameterization procedures. In the case of oil palm, this approach will provide breeders with physiological traits that may not be accessible by direct measurements, but are crucial for crop improving productivity.

Lastly, we would like to point out that carbon reserve status of palm family crops has the potential of providing diagnostic information on plant assimilate status and production potential. Such diagnoses can be extended spatially or into the future (extrapolation) with the help of models such as EcoPalm.

References

- Combres J-C., Mialet-Serra I., Bonnal V., Baron C., Flori A., Dingkuhn M., 2003 - EcoPalm, un modèle simple de simulation de la production mensuelle de cultures pérennes tropicales : exemple du palmier à huile. REA, 2^{ème} rencontres sur l'écophysiologie de l'arbre, La Rochelle, 8-12 décembre 2003, Communication orale.
- Dingkuhn, M., 2006 - Is plant growth driven by sink regulation? Implications for crop models, phenotyping approaches and ideotypes. *In: Gene-Plant-Crop Relations*, Wageningen UR FRONTIS Series, Springer (*in press*).
- Legros, S., Mialet-Serra, I., Caliman, J-P., Clément-Vidal, A., Siregar, F., Widiastuti, L., Jourdan, C. and Dingkuhn, M., 2006 – Carbohydrates reserves in 9 years old oil palm: Nature, Distribution and Seasonal changes, Communication IOPC 2006, Bali, 19 – 23 June 2006.
- Mialet-Serra, I., 2005 – Rôle et gestion des réserves carbonées face à la variabilité du climat chez une monocotylédone arborescente, le cocotier (*Cocos nucifera* L.) : Analyse et bilan. Th. : Ecophysiologie : ENSA de Montpellier, 112 p. + 6 annexes.
- Mialet-Serra I., Clément A., Sonderegger N., Roupsard O., Jourdan C., Labouisse J-P. & Dingkuhn M., 2005 - Assimilate storage in vegetative organs of coconut (*Cocos nucifera* L.). *Experimental Agriculture*, 41 (2): 161-174.
- Mialet-Serra, I., Clément-Vidal A., Roupsard O., Sonderegger N., Jourdan C., Labouisse J-P. & Dingkuhn M. (*in preparation*) – Role of carbon reserves in an arborescent monocot, the coconut palm (*Cocos nucifera* L.) in the face of climate variability: analysis and balance.
- Siregar, F., (*in press*) – Analysis of vegetative and fruit growths of adult oil palm: seasonal changes and applied treatments effects, ATP training report. 7p.



**PLANT GROWTH MODELS AND ENVIRONMENTAL STRESSES:
SHOULD WE BOTHER WITH CARBON?**

By Bertrand MULLER

Ecophysiologie des Plantes sous Stress Environnementaux, LEPSE, UMR759,
INRA - AGRO Montpellier, France.

Introduction

In many crop models (see CERES, GOSSYM, EPIC... e.g. Jones & Kiniry 1986), plant growth depends on the carbon entry into the plant either using explicit photosynthesis vs irradiance relationships or the Monteith equation that relates intercepted radiation to dry mass accumulation through integrated parameters. That plant growth on the long term (weeks) relies on energy capture and carbon accumulation is almost a tautology. However, on shorter terms, the importance of carbon is by far less clear with rather controversial elements in the literature. The picture could depend on the actual time scale : important factors determining growth at the minute to hour time scales may not be the same than at daily scales. It could also depend on the organ considered: some plant organs are obviously heterotrophic (such as roots, unmerged young leaves) and evidence suggest that their growth are more sensitive to carbon than emerged, illuminated leaves that have easier access to the C resource. Finally, a distinction should be done between growth in biomass and growth in length, surface or volume (which I will call expansion). Here I review some of the elements for the pro- and the contra- for a driving role of carbon on growth in relation to these distinctions. I also introduce new findings about the role of carbon in determining growth rates and/or plant growth responses to environmental stresses and finally propose to more widely use carbon status indicators to evaluate contributions of carbon economy to plant growth adjustments.

Uncoupling between leaf expansion and dry matter accumulation results in unstable specific leaf area that should not be used as (constant) parameter in growth models for single crops.

In most plant growth models, dry weight accumulation is related to incident radiation, energy capture efficiency (that relies on leaf area establishment and architecture) and conversion efficiency of light energy into dry matter. At the next step of the model, leaf area is classically inferred and fed back to the model from dry matter using specific leaf area as a parameter. When the aim of the model is to describe and predict variability in a large range of species and/or the behaviour of complex (eco)systems, taking specific leaf area as a parameter can be tenable since variability of specific leaf area between species is much higher than within species variability (Shiple *et al.* 2005). However, as shown by Tardieu *et al.* (1999) and Gunn *et al.* (1999), when one single species is considered, specific leaf area variation due to environment and/or developmental stage may be as high as variation between genotypes of the same species. This high variability is due to situations where environmental conditions have a differential effect on expansion rate and on photosynthesis. For instance, shaded plants have maintained expansion rate and decreased photosynthesis leading to increased specific leaf area whereas plants exposed to water deficit may have maintained photosynthesis and decreased expansion rate leading to decreased specific leaf area (Lecoeur *et al.*, 1995, Granier & Tardieu, 1999). These variations are not only due to changes in non structural carbohydrates but also to changes in tissue density (possibly cell wall material) and thickness (Gunn *et al.* 1999).

Plant growth as related to carbon availability

Emerged leaves of unstressed maize plants elongate at a constant rate that depends solely on the temperature of the elongating zone (Ben Haj Salah and Tardieu, 1995; Muller *et al.* 2001). Indeed, elongation rate at a hourly time step can be robustly related to temperature under a large range (8-50 mol/m²/j) of daily PPFs. By contrast, increasing the transpiration of these plants by increasing the leaf-to-air vapor pressure deficit will decrease elongation rate, probably via a hydraulic control (Ben Haj Salah and Tardieu 1996). It results that apparent changes of leaf elongation rate in growth chambers as lights are set on or off could be fully attributable to such changes in transpiration, not to incident light. Independence of expansion rate to incident light on the short term (hours) is confirmed in dicotyledons where relative expansion rate in 50% shaded plants is similar to that of control plants when the leaf is emerged and expands at a high rate (Granier and Tardieu, 1999), thus at a period when carbon demand is maximum. During this period, leaves are autotrophic so growing tissues are close to carbon source and leaf exert a high sink priority (Minchin *et al.*, 1993) and their expansion can be sustained even with low PPF.

In contrast with emerged, autotrophic leaves, leaf expansion rate of sunflower is severely reduced if a reduction in PPF occurs during the early stage of leaf development, during which expansion rate and presumably carbon demand are small (Granier and Tardieu 1999; Muller *et al.* 2001). In the same way, root elongation rate is closely dependent on intercepted PPF (Aguirrezabal *et al.* 1994). Beyond the fact that roots are heterotrophic by nature, the reason for such behaviour is that roots have a lower sink priority (as compared to growing leaves) because they are further from carbon source. It is noteworthy that roots (at least from annual crops) have a very limited storage capacity for carbon that corresponds to only few hours of autonomy (Muller unpublished). It has been proposed that the impact of carbon on the expansion of heterotrophic organs could be via an effect on cell division. Indeed, cell division is highly sensitive to the nutritional status of cells and C shortage is known to provoke cell cycle blockage at the G1/S and G2/M transitions (Van t'Hof 1968; Riou-Khamlichi *et al.* 2000). In maize roots, carbon limitation leads to parallel decrease of local sucrose and hexoses gradients and shortening of the meristem while activity of the meristem is maintained (Muller *et al.* 1998). In maize, lateral roots show a range of elongation rates that are related to meristem size and to sugar content (Muller unpublished).

In conclusion, expansion of heterotrophic organs (young leaves, roots) depends on carbon availability, possibly through a limitation of cell division. By contrast, the expansion of autotrophic is largely insensitive to irradiance when all climatic variables associated with changes in irradiance (temperature, leaf-to-air vapor pressure deficit) are carefully controlled. On longer terms (days), different PPFs may have a strong impact on leaf expansion and final leaf area either because (i) a reduction in young leaf relative expansion rate will have a permanent impact on absolute leaf expansion rate and thus final leaf area (Granier *et al.* 2006) or (ii) a reduction in leaf area triggered by shading will have a cascade effect on light interception and carbon assimilation by the plant (Chenu *et al.* 2005).

Is plant growth carbon limited under nutrient or water limitations?

Answering this question is central for designing models that best fit the behaviour of plants under nutrient or water limitations. Should these models include alterations of carbon-to-growth relationships within their engines or should they rely on totally different rationales based on carbon independent growth limitations? These later limitations could operate through (for instance) the hydraulic status of the plants, its redox status, the rheological properties of the cell wall, and could include several mediators such as stress hormones. It is thus desirable to both question the carbon

limitation and the occurrence of other limitations. Questioning other growth limitations is currently the matter of large set of studies I will not survey here (see examples of such studies in Voisin *et al.* 2006 and Muller *et al.* 2007). Questioning the carbon limitation can be performed by different strategies.

The first strategy is to study the interaction between carbon status and the environmental limitation. In other words, do the plants perform better with high carbon (most often PPFD or high CO₂) when they are exposed to nutrient or water limitation? The second possibility is to identify markers for the carbon dependency of growth under non stressful conditions and to see whether these markers reveal carbon dependency under stressful conditions. The third strategy is to demonstrate that a carbon driven model can account for plant growth responses to environmental stresses. To my knowledge, such a strategy has not yet been used and constitutes an avenue of research for plant modellers.

An example of the first strategy is followed by Wissuwa (2005) when questioning whether root growth of P starved plants is source or sink limited. An increased C entry into the plant through photosynthesis at high vs low light had no beneficial impact on growth at low P suggesting that root growth is sink- and not source limited. In accordance, a negative relationship was found between starch concentration (indicative of an excess of carbon) and growth.

An example of the second strategy is followed by Freixes *et al.* (2002) using arabidopsis plants that were exposed to a range of carbon stati obtained by changing the light intensity or the external sugar concentration supplied to the roots. When looking at the sugar concentration at the apical (growing) zone, these authors found a robust positive relationship between hexose concentration and root elongation rate for both primary and secondary roots. Such a relationship could also account for plant to plant variability within treatments as well as variability induced by a mutation in the starch synthesis pathway leading to plants with much altered sugar content (Freixes 2003). These relationships were progressively lost when plants were exposed to conditions favouring sink limitation of growth: very high external sugar concentrations or very low light intensity. These relationships were also progressively lost when the plants were exposed to increasing water deficit suggesting a progressive passage of source- to sink- limitation of root growth under water deficit (Freixes 2003). The significance of the sugar to growth relationship could be related to either the substrate nature of sugars that trigger the functioning of the enzymes of its metabolism or to the signaling role of sugars, in particular their role on the enzymes of their own metabolism. Interestingly, it was shown (Bläsing *et al.* 2005) that starchless mutant *pgm* showing much lower sugar levels at the end of the night and much higher sugar levels at the end of the day have gene expression that is much more strongly affected at the end of the night than at the end of the day suggesting a more important signaling role of famine signals (when there is too less sugars) than feast signals (too much sugars).

Another example of the second strategy is given by recent studies from Stitt's group at Golm. Using robotized assays (Gibon *et al.* 2004), they have evaluated a wide range of metabolites and enzyme activities related to C metabolism in a collection of arabidopsis ecotypes under unstressed conditions. The main output of this impressive study is that a negative relationship was found between growth and starch accumulation and a positive relationship was found between growth and some enzymes, in particular Asp amino transferase and the Krebs cycle enzyme fumarase, suggesting fast growing arabidopsis ecotypes are those that have the capacity to rapidly generate ATP through the Krebs cycle and to tightly connect N and C metabolisms through amino transferases (Cross *et al.* 2006). It remains that such studies should now be performed under stressfull conditions in order to evaluate if such relationships remain.

Conclusions

The role of carbon into models is most often central although there is evidence that organ expansion can be often sink- and not source- limited, depending on organ and time scale. When exposed to environmental stresses, there is growing evidence that source limitation may be replaced by yet unidentified sink limitation. In this context, major challenges for the researchers are (i) to carefully built up models that will be able to account for progressive changes of source to sink limitations of organ growth, depending on environmental conditions (ii) to define new biochemical markers for carbon status that can be used to identify situations of carbon limitation.

References

- Aguirrezabal L.A.N., Deléens E., Tardieu F. (1994) Root elongation rate is accounted for by intercepted PPFD and source-sink relations in field and laboratory-grown sunflower. *Plant, Cell and Environment* 17, 443–450.
- Ben Haj Salah H, Tardieu F. 1995. Temperature affects expansion rate of maize leaves without change in spatial distribution of cell length. Analysis of the coordination between cell division and cell expansion. *Plant Physiology* 109: 861-870.
- Ben Haj Salah H, Tardieu F. 1996. Quantitative analysis of the combined effects of temperature, evaporative demand and light on leaf elongation rate in well watered field and laboratory grown maize plants. *Journal of Experimental Botany* 47: 1689-1698.
- Bläsing OE, Gibon Y, Günther M, Höhne M, Morcuende R, Osuna D, Thimm O, Usadel B, Scheible WR, Stitt M (2005) Sugars and Circadian Regulation Make Major Contributions to the Global Regulation of Diurnal Gene Expression in Arabidopsis *The Plant Cell*, Vol. 17, 3257–3281
- Chenu K, Franck N, Dauzat J, Barczy JF, Rey H, et Lecoeur J. 2005. Integrated responses of rosette organogenesis, morphogenesis and architecture to reduced light in Arabidopsis thaliana results in optimized light interception. *Functional Plant Biology* 32.
- Cross, J.M., Von Korff M., Altmann, T., Bartzetko, L., Sulpice, R., Gibon, Y., Palacios, N., and Stitt M., 2006 - Variation of Enzyme Activities and Metabolite Levels in 24 Arabidopsis Accessions Growing in Carbon-Limited Conditions. *Plant Physiol.* 142: 1574-1588.
- Freixes S, Thibaud MC, Tardieu F, Muller B (2002) Root elongation and branching is related to local hexose concentration in Arabidopsis thaliana seedlings. *Plant, Cell and Environment*, 25, 1357-1366.
- Gibon, Y., Bläsing, O.E., Hannemann, J., Carillo, P., Höhne, M., Hendriks, J.H., Palacios, N., Cross, J., Selbig, J., and Stitt, M. (2004). A robot-based platform to measure multiple enzyme activities in Arabidopsis using a set of cycling assays: Comparison of changes of enzyme activities and transcript levels during diurnal cycles and in prolonged darkness. *Plant Cell* 16, 3304–3325.
- Granier C, Aguirrezabal L, Chenu K, Cookson SJ, Dauzat M, Hamard P, Thioux JJ, Rolland G, Bouchier-Combaud S, Lebaudy A, Muller B, Simonneau T, Tardieu F (2006) PHENOPSIS, an automated platform for reproducible phenotyping of plant responses to soil water deficit in Arabidopsis thaliana permitted the identification of an accession with low sensitivity to soil water deficit *New Phytologist* 69: 623–635.
- Granier C, Cookson SJ, Tardieu F, Muller B (2006) Cell cycle and abiotic stresses. In, Cell cycle control and plant development. Blackwell Edition, in press.

- Granier C., Tardieu F. 1999. Leaf expansion and cell division are affected by reduced intercepted light but not after the decline in cell division rate in the sunflower leaf. *Plant, Cell and Environment*, 22, 1365-1376.
- Gunn S, Farrar JF, Collis BE, Nason M (1999) Specific leaf area in barley: individual leaves versus whole plants. *New Phytologist* 143: 45-51.
- Jones C.A. & Kiniry J.R. (1986) CERES-Maize. A simulation model of maize growth and development. Texas A&M University Press. College station.
- Lecoeur J, Wery J, Turc O, Tardieu F. 1995. Expansion of pea leaves subjected to short water deficit: cell number and cell size are sensitive to stress at different periods of leaf development. *Journal of Experimental Botany* 46: 1093-1101.
- Minchin PEH, Thorpe MR, Farrar JF. 1993. A simple mechanistic model of phloem transport which explains sink priority. *Journal of Experimental Botany* 44: 497±955.
- Monteith JL. 1977. Climate and the efficiency of crop production in Britain. *Philosophical Transactions of the Royal Society. London, B*, 281: 277±294.
- Muller B, Bourdais G, Reidy B, Bencivenni C, Massonneau A, Rolland G, Conéjéro G, Rogowsky P Tardieu F(2007) Association of specific expansins with growth of maize leaves is maintained with environmental, genetic and developmental sources of variation. *Plant Physiol* (in press).
- Muller B, Reymond M, Tardieu F (2001). The elongation rate at the base of a maize leaf shows an invariant pattern during both the steady-state elongation and the establishment of the elongation zone. *J Exp Bot* 52, 1259-1268.
- Muller B, Stosser M, Tardieu F (1998). Spatial distributions of tissue expansion and cell division rates are related to irradiance and to sugar content in the growing zone of maize roots. *Plant Cell Environ.* 21: 149-158.
- Riou-Khamlichi C, Menges M, Healy JMS, Murray JAH (2000) Sugar control of the plant cell cycle: differential regulation of Arabidopsis D-Type Cyclin gene expression. *Molecular and Cellular Biology* 20(13): 4513-4521.
- Riou-Khamlichi C., Menges M., Healy J.M., Murray J.A. (2000) Sugar control of the plant cell cycle: differential regulation of Arabidopsis D-type cyclin gene expression. *Molecular and Cellular Biology* 20, 4513–4521.
- Shiple B, Vile D, Garnier E, Wright IJ, Poorter H (2005) Functional linkages between leaf traits and net photosynthetic rate: reconciling empirical and mechanistic models. *Functional Ecology* 19:4, 602-615.
- Tardieu F, Granier C, Muller B (1999). Modelling leaf expansion in a fluctuating environment : Should we use equations describing carbon budget, tissue expansion or cell division ? *New Phytol*, 143, 33-43.
- Van't Hof J. (1968) Control of cell progression through the mitotic cycle by carbohydrate provision. *Journal of Cell Biology* 37, 773-780.
- Voisin AS, Reidy B, Parent B, Rolland G, Redondo E, Gerentes D, Tardieu F, Muller B (2006) Are ABA, ethylene or their interaction involved in leaf growth response to soil water deficit ? An analysis using naturally

occurring variation or genetic transformation of ABA production in maize. *Plant Cell Environ* 29: 1829-1840.

Wissuwa M, Gamat G Ismail AM (2005) Is root growth under phosphorus deficiency affected by source or sink limitations? *Journal of Experimental Botany*, Vol. 56, No. 417, pp. 1943–1950, July 2005.



SOME EXAMPLES OF PLANT FUNCTIONING MODELS

MODELLING CARBON ALLOCATION AND FRUIT QUALITY IN PEACH TREES

By Michel GENARD, Françoise LESCOURRET & Nicolas MOITRIER
RU 1115 Plantes et Systèmes de culture Horticoles, INRA, 84000, Avignon.

Introduction

Carbon allocation within a plant depends on complex rules linking source organs (mainly leaves) and sink organs (mainly roots and fruits) for assimilates. The complexity comes essentially from regulations due to feedback mechanisms and interactions between different functions. In order to analyse this complexity, carbon models of plant growth have been developed during the last thirty years. These models are powerful tools to analyse how source and sink number, size and position within the plant affect the carbon partitioning and as a consequence the vegetative and reproductive growth, and the fruit quality.

The first objective of this short presentation is to address the conceptual framework of carbon allocation in plant based on the current theories. The second objective is to exemplify the interest of carbon partitioning modelling using a *PEACH* plant model to assess the effect of management practices such as fruit thinning and plant pruning on vegetative growth, fruit production and quality.

Theories of carbon allocation

As stated by Lacoite (2000), Leroux *et al.* (2001) and Thornley and Johnson (1990), four main approaches have been used in models of carbon economy. These approaches are either empirical and allometric, teleonomic, source-sink, or based on transport and chemical/biochemical conversion concepts.

The models of carbon balance based on empirical allocation coefficients give usually reasonable predictions in the range of conditions for which these coefficients have been measured.

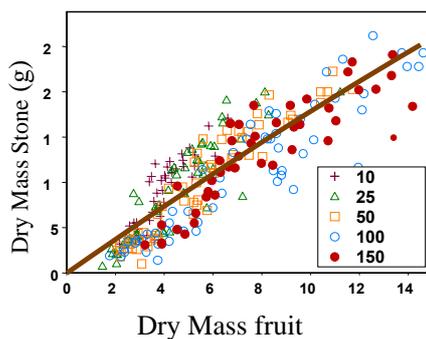


Figure 1- Allometric relationships between stone mango dry weight and fruit dry weight. Five different leaf to fruit ratio were used (from Léchaudel *et al.*, 2002)

An example is given for mango fruit for which there is a strong allometric relationship between stone dry weight and fruit dry weight (Figure 1). Derivation of such an allometric equation has been used by Lescourret and Génard (2005) to partition carbon between flesh and stone in a model of virtual peach fruit.

Many models of plant carbon allocation are based on teleonomic approaches where some goal is assumed, e.g. a functional balance between shoot and root is assumed in the peach model. In this

case, a variable RS reflecting the balance between the mass of roots younger than one year (Wr) and the leafy shoot mass (Ws) is defined as:

$$RS = \frac{I}{RSe} \frac{Wr}{Ws}$$

The parameter RSe is equal to the ratio of weight of young roots and weight of shoots when the tree is at equilibrium. When RS is greater than one, there is an imbalance in favour of roots and assimilates are preferentially allocated to shoots, whereas when RS is less than one, assimilates are preferentially allocated to roots. The consequence is a fluctuation of the root:shoot ratio along the season. Equilibrium is reached when RS is equal to one.

In the source-sink relationships-based models, the carbon allocation is assumed to depend on the respective ability of the different sinks to import available assimilates from the sources. This ability or "sink strength" or "sink demand" is based on the genetically determined potential growth.

Experimentally determined seasonal patterns of organ growth potential are frequently used in the literature to represent potential net sink strengths. However, this idea implies that constrained fruit growth is able to reach the potential growth rate after removal of competing sinks. This capability was not observed for peach. Lescourret *et al.* (1998) proposed an equation for sink demand which emphasizes the role of fruit history by means of the accumulated growth (W), both in terms of sink size and sink activity.

An important step after the quantification of organs demands is the carbon allocation from sources to sinks. When the demand is less than the supply, each sink gets its own demand and the excess supply goes to reserves. On the other hand, there are two approaches to deal with the case of supply shortage. In the "proportional" approach, the carbon supply from sources is shared by the sink organs which get each the same proportion of their demand. Alternatively, in the "hierarchical-priority" approach, the sink with the highest priority is served first, then the component with the next priority level is considered, and so on. The maintenance respiration requirements are assigned the highest priority because they are vital for the organ survival. Our peach model uses a mixture of these rules.

Models based on transport and chemical/biochemical conversion concepts opened the way for a mechanistic description of the carbon partitioning. They made it possible to avoid the empirical allocation coefficients, functional balance rules, or fixed allometric relationships. Such concepts are used in our peach model in order to simulate the C translocation between organs within the plant. The C unloading to the fruit and the C compartmentation within the fruit between the different species of sugars are two important aspects for models focusing on fruit quality.

The sugars can be unloaded from the phloem to the fruit by active transport, mass flow or diffusion. Fishman and Génard (1998) proposed the following equation to represent the total uptake of carbohydrates (U) by the fruit:

$$U = Ua + (1 - \square p) * ((Cp + Cf) / 2) * Up + A * ps * (Cp - Cf)$$

where Ua is the rate of uptake due to active transport obeying a Michaelis-Menten equation, Up is the phloem flow of liquid entering the fruit, Cp and Cf are the sugar concentrations in the phloem and fruit, respectively, $\square p$ is the reflexion coefficient which is a measure of impermeability of the cell membrane to the solute, A is the membrane area through which the solutes diffuse and ps is the solute permeability coefficient. If $\square p = 1$ the membranes are impermeable and there is no sugar uptake through mass flow. As ps is usually small, the diffusion component can often be neglected. The rate (Ua) obeys the Michaelis-Menten equation:

$$Ua = vm Cp / (KM+ Cp)$$

where vm is the maximum uptake rate and KM is the Michaelis-Menten constant.

The transformation of phloem sugars (sucrose, sorbitol,..) into sink soluble carbohydrates (sucrose, glucose, fructose,...), starch or cell walls is one aspect of carbon partitioning that is usually ignored. It is a major process of growth because sink soluble carbohydrates drive the sink osmotic potential which in turn drives the sink water uptake. When a sink is harvested for its quality, as in the case of fruits, the transformation of phloem sugars into other sugars, and cell walls components determines their quality which is an important component of their market value.

Génard and Souty (1996) and Génard *et al.* (2003) designed a mechanistic model, called SUGAR, to predict the changes in sugar composition during fruit development (Figure 2).

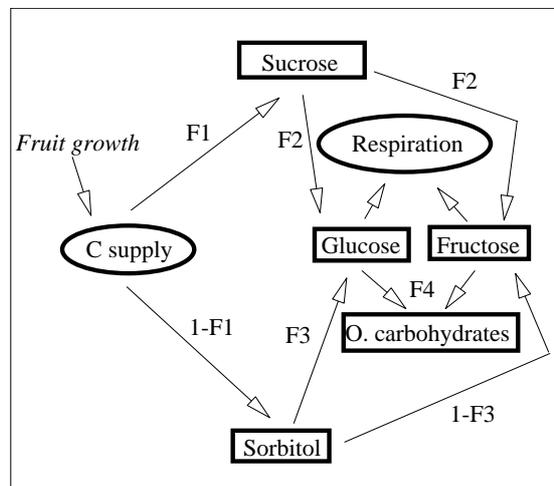


Figure 2- Diagram illustrating the carbon partitioning by the SUGAR model to predict the changes in sugar composition along the peach fruit development. Arrows, ellipses and boxes represent carbon flows, carbon supply and losses, and carbohydrate compartments, respectively. The F_i are allocation functions.

The model was designed for peach, but the main principles can be used for other fruits. In this model, the unloaded sugars are either directly stored in the tissues or transformed in CO_2 through the respiration process or transformed into other sugars or used to synthesize other compounds (structural carbohydrates, etc.). The enzymatic reactions were described according to the "rate law" of chemical kinetics, which states that the rate of a reaction is proportional to the reactant concentration. Thus, the rates of change in the amounts of carbon in sugar compounds depend on sugars already accumulated in the fruit flesh. They are described through a set of differential equations, each equation being of the following form:

$$\frac{dC_j}{dt} = E_j + \sum_{i \neq j} k_{ij}(\theta, x) C_i - C_j \sum_{i \neq j} k_{ji}(\theta, x) - R_j$$

where C_j is the carbon amount in sugar j , E_j and R_j , which can be equal to zero, depending on the compartment, are respectively the carbon flow from the phloem and the carbon loss by respiration, k_{ij} is a function of parameter (θ) and variable (x) describing the relative rate of sugar transformation of sugar i into sugar j .

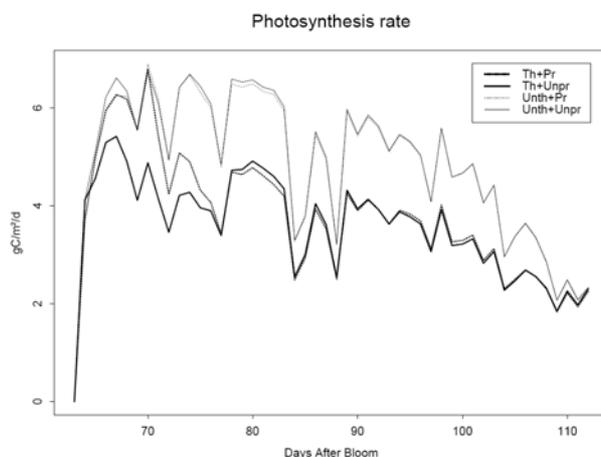
Effect of cultural practices

Our peach model describes carbon transfer within the plant between shoots bearing fruits, trunk and branches, and roots. The model assumes that the plant is a set of shoots bearing fruits connected to each other by the branches. The carbon allocation between shoots bearing fruits and with the other parts of the plant is based both on source-sink concepts and a simplified version of the Munch transport theory. The leafy shoots have the first priority for growth. The balance between root and shoot growth is managed using the principles of "functional equilibrium". The physiological processes considered are the leaf and fruit photosynthesis, the respiration of all the plant organs, the carbon storage and remobilisation in leaves, branches-trunk and roots, the growth of the organs. The leaf photosynthesis is regulated by their reserve concentration. The fruit quality traits are the fruit mass, the percentage of flesh and the concentrations of sucrose, sorbitol, glucose and fructose within the flesh. These concentrations can be used to calculate the sweetness of the fruit.

Simulations were performed from 63 to 111 days after bloom, on a peach tree with two main branches, 20 shoots bearing fruits, 188 fruits and 119 shoots before pruning and thinning. The effect of summer pruning and fruit thinning was analysed considering an unpruned high loaded tree, an unpruned thinned tree (80% of fruits removed), a pruned (50% of leafy shoots removed) high loaded tree, and a pruned thinned tree (Figure 3).

The photosynthesis rate was the highest for high loaded trees. The pruned thinned tree exhibited also a high photosynthesis rate during the 10 first days of simulation, and a decreased rate when the tree recovered its foliage area. Pruning and thinning had both an effect on growth and reserve accumulation of the different organs. However, the effect was very different on sinks and source organs. The sink organs grew more and accumulated more reserves when the leaf:fruit ratio was greater. The fruits accumulated more sugars and were sweeter. The shoots grew more when the tree was pruned and, for a given pruning intensity, they grew more when the tree was thinned. The effect of pruning on shoot growth can be interpreted according to the coordination theory. But why do shoots grow more after thinning when the model assumes they have a priority for carbon? The analysis of simulations shows that there is more carbon available for roots when the tree is thinned. The subsequent increase of root growth induces an increase of shoot sink strength due to the application of the "functional equilibrium" theory.

A



B

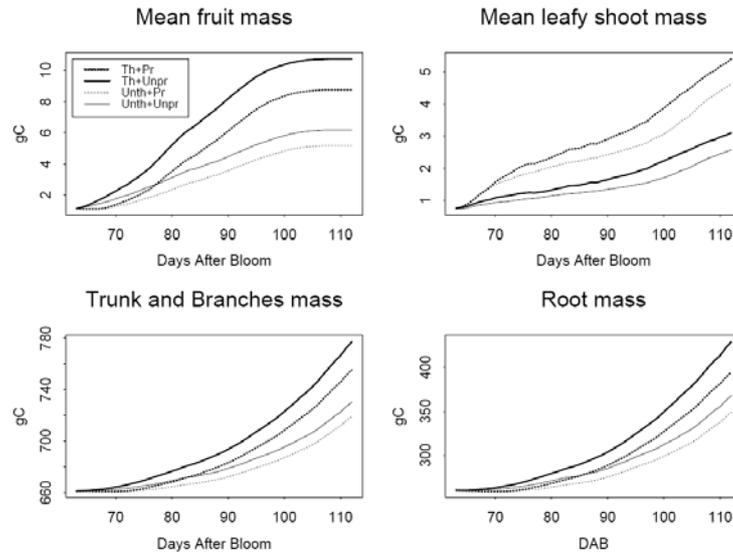


Figure 3- Peach leaf photosynthesis rate (A) and carbon partitioning between fruits, leaves, aerial wood and roots (B) as predicted by the virtual tree for different levels of fruit thinning and summer pruning. The treatments are unpruned unthinned tree (Unpr+Unth), unpruned thinned tree (80% of fruits were removed, Unpr+Th), a pruned (50% of leafy shoots were removed) unthinned tree (Pr+Unth), and a pruned thinned tree (Pr+Th).

In most of the published studies, the negative effect of high fruit load on shoot growth is interpreted as a direct competition between fruits and shoots. Our simulation leads to an alternative interpretation of the fruit load effect on vegetative growth: the competition between fruits and roots for carbon decreases both the fruit and root growth, but the decrease of shoot growth only results from the decrease of root growth.

Conclusions

Carbon partitioning in plants is controlled by a number of factors which include photosynthesis, the number and location of competing sinks, storage capacity and vascular transport. Although there is considerable information on individual processes in plants such as photosynthesis, translocation and cell growth, it appears that the control of carbon partitioning at the whole plant level is still poorly understood. Indeed, many processes are closely interrelated and more integrative research work based on modelling approaches is needed. One interesting way is to follow the teleonomic approach which assumes that the different processes are interrelated through a common goal. Another way is to integrate basic knowledge in source-sink and transport and chemical/biochemical models able to link the different processes. Such models are based on different theories corresponding to more or less accurate description of mechanisms. They allow to take into consideration the regulation of processes and their interactions. They are able to simulate complex allocation processes and could be useful tools to analyse the genetic variability. With the increasing power of computers and informatic languages, it becomes possible to link plant architecture and function in functional-structural plant models which opens new avenues to link plant growth and development with environmental and management factors.

References

- Fishman S., Génard M. 1998. A biophysical model of fruit growth: simulation of seasonal and diurnal dynamics of mass. *Plant, Cell and Environment* 21, 739-752.
- Génard M., Lescourret F., Gomez L., Habib R. 2003. Changes in fruit sugar concentrations in response to assimilate supply, metabolism and dilution: a modeling approach applied to peach fruit (*Prunus persica*). *Tree Physiology* 23, 373-385.
- Génard M., Souty M. 1996. Modeling the peach sugar contents in relation to fruit growth. *Journal of the American Society for Horticultural Science* 121, 1122-1131.
- Lacointe A. 2000. Carbon allocation among tree organs: a review of basic processes and representation in functional-structural tree models. *Annals of Forest Science* 57, 521-533.
- Léchaudel M., Génard M., Lescourret F., Urban L., Jannoyer M. 2002. Leaf-to-fruit ratio affects water and dry-matter content of mango fruit. *Journal of Horticultural Science and Biotechnology* 77, 773-777.
- LeRoux X., Lacointe A., Escobar-Gutierrez A., Le Dizes S. 2001. Carbon-based models of individual tree growth: a critical appraisal. *Annals of Forest Science* 58, 469-506.
- Lescourret F., Ben-Mimoun M., Génard M. 1998. A simulation model of growth at the shoot-bearing fruit level. I. Description and parameterization for peach. *European Journal of Agronomy* 9, 173-188.
- Lescourret F., Génard M. 2005. A virtual peach fruit model simulating changes in fruit quality during the final stage of fruit growth. *Tree Physiology* 25, 1303-1315.
- Thornley J. H. M., Johnson I. R. 1990. *Plant and crop modelling*. Oxford University Press, Oxford, 669 p.



Cf. Annexe

**ECO PALM: A SIMPLE, PHYSIOLOGICAL MODEL
TO SIMULATE MONTHLY VARIATION OF FRUIT PRODUCTION OF AN ADULT OIL PALM PLANTATION**

By Jean-Claude COMBRES, Isabelle MIALET-SERRA, Vincent BONNAL,
Albert FLORI and Michael DINGKUHN.

RU 59, "Integrative Modeling", CIRAD, AMIS department, Montpellier, France.

Introduction

A major scientific challenge that has evolved during the past decade is to understand, simulate and predict the seasonal variations of bunch production in oil palm plantations. In Ivory Coast, monthly yield of fresh fruit bunches (FFB) varies from 0.05 to 0.2 t ha⁻¹ month⁻¹ in July, August and September, to 2.5 to 6.6 t.ha⁻¹.month⁻¹ in March and April for L2T x D10D genetic material. Variation and level of monthly production have implications for oil palm industry and management of plantations. This has motivated numerous authors to model and simulate production according to environment and climate.

Early attempts in crop modelling tried to predict annual FFB production which varied from 9 to 22 t ha⁻¹ year⁻¹ FFB in Ivory Coast and from 6 to 26 t ha⁻¹ year⁻¹ FFB in Indonesia. The first models were statistical (Ong 1982, 1983). Dufour *et al.* (1988), showed the importance of (i) water deficit at different phases of sensitivity between 1 and 45 months before harvest of a given bunch, and (ii) effective solar radiation. The second generation of models simulated canopy CO₂ assimilation. The OPSIM model was implemented by Van Kraalingen (1985) and Van Kraalingen, Breure & Spitters (1989) on the base of the generic SUCROS model (van Keulen, 1982). OPSIM considered a potential production only and thus had no water balance module. Dufrêne (1989) introduced a semi-empirical water balance for the model SIMPROD. In this model, growth rate of different vegetative parts can be adjusted independently according to empirical rules for assimilate demand and partitioning. In these two models, photosynthesis, growth and maintenance respiration and the plant-level carbon balance are simulated quantitatively according to generic, well-documented coefficients. Excess of assimilates, after partition to vegetative growth, are accumulated in a pool of carbohydrates and converted at the end of the year to FFB production.

Several tentative where made to modify these models to operate at monthly time step (Henson, 1997) and to evaluate alternative mechanisms that may explain seasonal variation, which is usually very large (Henson, 1999). Nevertheless, seasonal variation remains largely unexplained (Henson, 2004). The determinism of intra-annual variation in oil palm production is complex because it is obviously driven not only by carbon assimilation but also phenology, despite the fact that production occurs throughout the year. The principal component of variation between months of FFB production is the number of bunches (relative variation up to 1:30). In adult palm plantations there is also a seasonal variation of mean bunch weight (variation up to 1:3).

Hypotheses of EcoPALM model

The ECO PALM model presented here introduces two new concepts in an attempt to improve simulation of seasonal production of oil palm, (i) phytomer-level phenology integrating photoperiodic responses and thermal time, and (ii) feedbacks of assimilate sink-source relations on phenology and reproductive sinks. These concepts are applied to the L2T x D10D hybrid grown in Ivory Coast. We will focus on the innovative components of the model and will not go into the details of simulation of

carbon assimilation and water balance which use established concepts and formalisms (SARRA-H crop model: Dingkuhn *et al.* 2003; Sultan *et al.*, 2005).

Adult Palm plantations (10 to 20 years) are considered to have constant Leaf Area Index (LAI) and constant, vegetative dry matter production (VDMP). Drought or climatic factors, however, can reduce VDMP, and the assimilate demand for VDMP varies according to maintenance respiration, which depends on temperature. The genotypic, annual rate of leaf production is assumed to be constant in terms of thermal time. These assumptions are based on the general observation that oil palm, and other Arecaceae, has very little phenotypic plasticity of the vegetative apparatus, as indicated by relatively constant leaf (palm) dimensions and general architecture (coconut: Mialet-Serra *et al.*, 2005; unpublished data on oil palm are available). Consequently, fluctuations in carbon assimilation mainly bear on bunch production and much less on vegetative maintenance and growth.

Monthly bunch yield is assumed to be generated during the month preceding maturity (and harvest). This is a simplification because bunches are grown during 10 months, but the bulk of their demand for assimilates occurs during the final phase (oleosynthesis, a costly process). The inflorescences are thus the most variable sink, but they do not act as a simple spill-over compartment. They are simulated as demand functions dimensioned individually according to phenological rules and feedbacks from inter-sink competition. Temporary disequilibria between plant-level, aggregate sources and sinks are buffered by (i) transitory storage (Mialet-Serra *et al.*, 2005) and (ii) feedback inhibition of carbon assimilation (Dingkuhn *et al.*, 2006).

Sensitivity to day length: The potential number of bunches per month is determined by the number of phytomers (leaves and inflorescences) initiated, and whose final phase of development falls into the month in question. Potential bunch number is attained if sex ratio is 100 % female and if no inflorescence abortion occurs.

Leaf (phytomer) initiation has constant plastochron. Assuming that growth and development rate have the same cardinal temperatures, base temperature is 16.25 °C Henry (1957). The thermal time between the initiations of two leaves is calibrated on mean annual leaf emissions and estimated at 165 °C.d. The seasonal variation of temperature induces a slight variation of number of leaves initiated every month.

The time of leaf initiation is unknown, but it occurs before the formation of the leaf primordia becomes visible by dissection and microscopy, at about rank -50 to -60 (counting backwards from appearance on the crown; Corley and Tinker 2003). Consequently, leaf initiation may occur 48 to 50 months before maturity of the bunch it carries on its axil.

The number of bunches harvested each month is sometimes superior to the maximum number of leaves that initiated per month (figure 1). This phenomenon can be explained by a photoperiod-sensitive phase (PSP) during inflorescence development. This process would operate at phytomer level and not at whole-plant level. The total duration between leaf initiation and flowering varies according to day length during PSP. Since the rhythm of phytomer production is constant and that of flowering is not, inflorescences ready to flower queue up during periods of unfavourable day length or photoperiod (not to be confounded with solar radiation), whereas peaks of flowering events occur when the opposite is the case. According to the model and observations on the genotype studied here, the peak of bunch harvests occurs in April to March in the northern hemisphere and in October to November in the southern hemisphere, but other genetic materials may respond differently and require different model parameters. The photoperiodic concept was applied in this model because no

known climatic variable appears to explain oil palm flowering patterns equally well, and because photoperiodism occurs in virtually all plants to variable degrees.

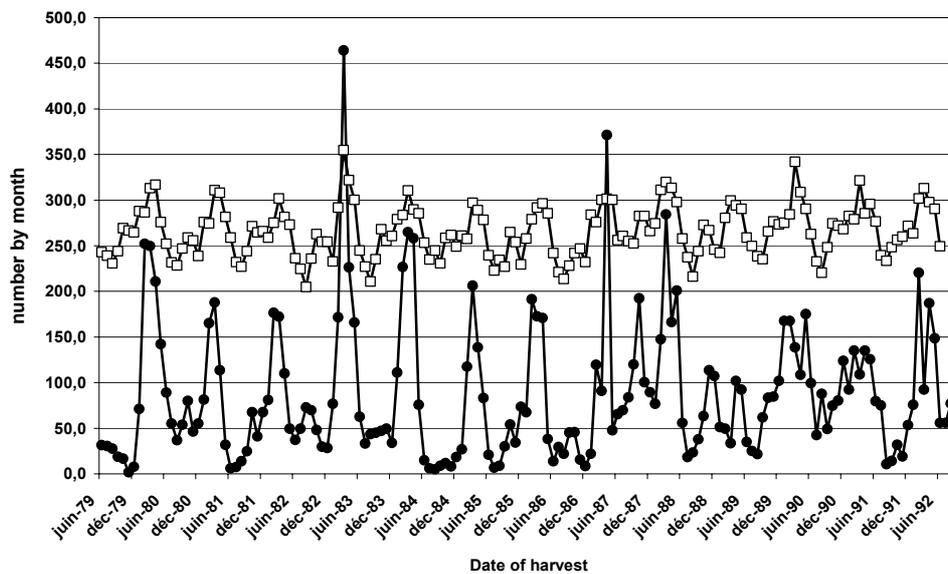


Figure 1-Number of bunches harvested (full circle) and number of leaves initiated 49 months before (open square)

The index of internal competition (I_c). I_c is a state variable describing the ratio between aggregate source and sink capacity at any given time at the plant scale, determines the sex of inflorescences and their abortion during specific, sensitive phenological phases. The general concepts used in ECOPALM is close to that described for the EcoMeristem model (Luquet & al. 2006), applied to the production of assimilates (supply) and the different sinks (demand) scale of a plant population and canopy. The various sinks are the maintenance respiration, vegetative growth and associated growth respiration, and growth of the inflorescences and bunches and associated growth respiration, the latter being particularly high during oil synthesis. Values of $I_c < 1$ trigger adaptive adjustments in plant organogenesis and morphogenesis, resulting in phenotypic plasticity. For oil palm, only the reproductive sinks are assumed to be plastic. Three types of adjustments can occur under assimilate deficiency, (i) decrease in initial sex ratio (ii) abortion of inflorescence during sensitive phases, and (iii) bunch weight decrease and bunches failure in extreme cases.

Storage of assimilates. When $I_c > 1$, the excess assimilates are reversibly stored as reserves, which are known to be mainly located in the trunk. End product inhibition of photosynthesis occurs when the reserve compartment is saturated. Assimilate deficiency ($I_c < 1$) is partially compensated by reserve mobilization, while taking into account the cost of mobilization and transport.

The potential reproductive demand is the product of potential bunch weight and number of bunches one month before bunch maturity. If there is assimilate deficiency during fruit filling, the yield decreases according to the rules of the use of the carbon reserves (hypothesis of partial satisfaction of deficit).

Description of EcoPALM model

The model operates at two time steps, daily and monthly. The water and the carbon balance are implemented daily using daily agro-meteorological data. For all other processes, the model uses monthly accrued values for state variables. Model runs are executed between two dates of simulation and require at list five years of agro-meteorological data prior to the period for which yield is simulated. This is necessary because the model operated with the concept of long-term (pluri-annual) effects of carbon balance history (*Ic* dynamics).

Each model run consists of a pre-run determining phenology and organogenesis, and a main run determining growth and resource dependent sexualization, abortion and fruit filling.

Pre-run. Upon initialization, the model computes the number of leaves (phytomers) by hectare whose inflorescence can potentially (if sex ratio is 100% and no abortions occur) attain maturity for any given month of simulation. The state variable *PNB* quantifies the Potential Number of Bunches. For each phytomer, the model computes by summation of thermal time the date of the beginning of PSP ($STTbPSP$ = duration from initiation to PSP onset), the date of end of PSP (using an algorithm involving day length and thermal time), and the date of harvest ($STTaPSP$ = duration from end of PSP to maturity). Then, the model identifies the phytomers that contribute to the plant population based harvest for each month of simulation, by taking into account the number of leaves initiated by hectare (*PNB*) population density (figure 2).

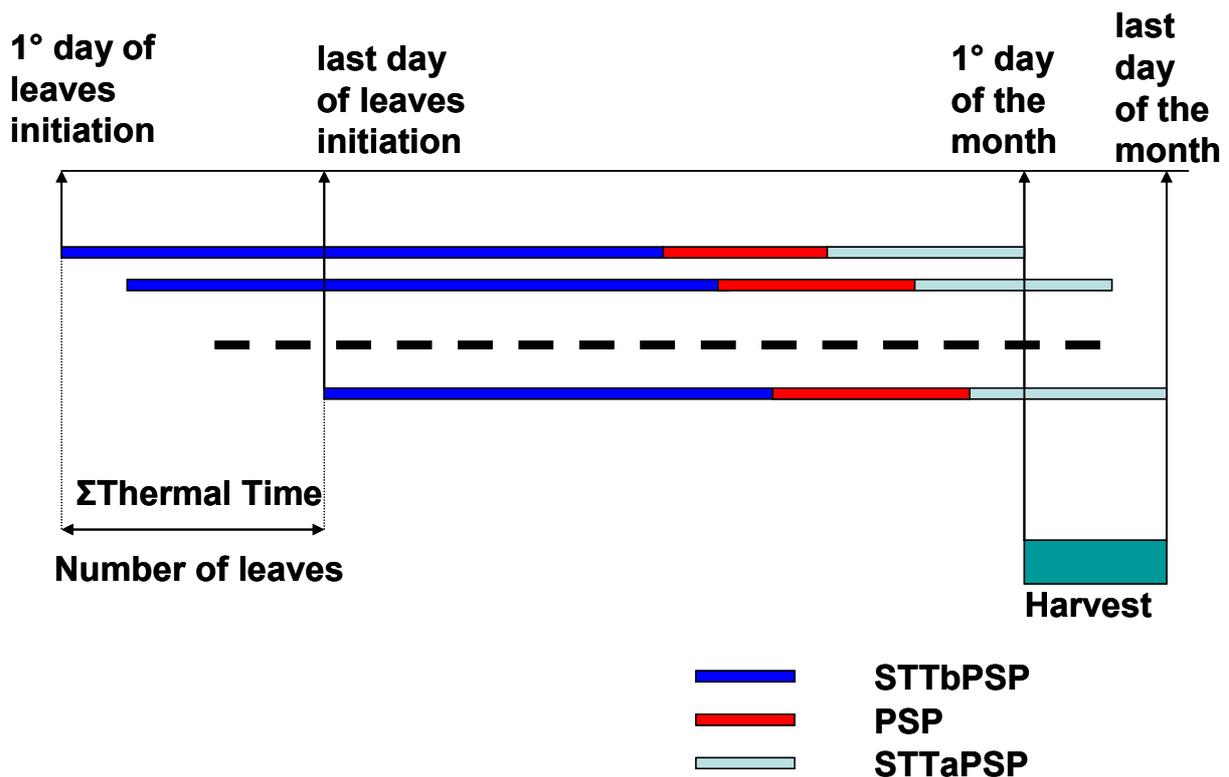


Figure 2- Schematic phenology and estimation of potential number of bunches equal to number of leaves initiated reaching harvest during the same month.

To compute the duration of PSP, the model uses a very simple routine developed for short-day plants. Every day, if day length (*DL*) is superior to a critical value (*PPcrit*), we calculate the effective photoperiod (*PPeff*) with $PPeff = \max(0; DL - PPcrit)$. The development rate (*V*), or rate of progress to flowering, is proportional to the reciprocal of the effective photoperiod. The end of PSP (corresponding to panicle initiation of cereals) occurs when the accumulation of the development

increments attains threshold value (PP_{seuil}). This module has four calibration parameters: $STTbPSP$, $STTaPSP$, PP_{crit} , PP_{seuil} . For simplicity, we assume that PSP ends at flowering.

Main run. No detail is provided here on the algorithms of light interception, carbon assimilation, respiration, transpiration and water deficit. For the understanding of the specificities of this model it is necessary, however, to explain the feedbacks of assimilate status (Ic) on yield formation. In principle, any reproductive process can be subject to Ic feedbacks at any developmental stage of a given phytomer. We empirically identified 4 Ic -sensitive phases of inflorescence and bunch development. The first is the determination of initial sex ratio; the three others cause abortions of female inflorescences when carbohydrates supply is insufficient ($Ic < 1$). From several trials of simulation, we found that the variation of Ic is more effective than absolute Ic for the prediction of the initial sex ratio. ECOPALM computes $RatioIc = Ic_{month} / average(Ic_{month-1}; Ic_{month-2})$ and the Initial Sex Ratio (ISR) is simulated as $ISR = a + b * RatioIc$. The parameters a and b are found by calibration using statistical parameter optimization. The Initial Number of female Inflorescence ($INFI$) and the number of male inflorescences (NMI) are obtained from PNB and ISR as $INFI = PNB * ISR$ and $NMI = PNB * (1 - ISR)$.

The abortion rate for each successive phase (AR_{phase}) varies between 0 and 1 as a linear function of Ic_{phase} , provided that Ic_{phase} is inferior critical Ic_{tph} :

$$1 - AR_{phase} = \min(1; \min(1; Ic_{phase}) / Ic_{tph}).$$

The number of bunches (NB) harvested each month is therefore:

$$[NB = PNB * ISR * (1 - AR_{phase2}) * (1 - AR_{phase3}) * (1 - AR_{phase4})]_{month}$$

The actual sex ratio (ASR) for each month is:

$$ASR_{month} = [ISR * (1 - AR_{phase2}) * (1 - AR_{phase3}) * (1 - AR_{phase4})]_{month}$$

$$ASR_{month} = NB_{month} / PNB_{month}$$

The potential bunch weight (PWB) is a genotype dependent crop parameter. During bunch development this potential could be reduced to maximum bunches weight (MBW) by Ic dependent adjustments of the sink, which reduce flower number and the number of fruits per flower. The actual, final bunch weight (OBW) can again be inferior to MBW if assimilates supply is insufficient during fruit filling. This latter process is not a sink adjustment but a limitation to sink satisfaction (filling).

The ratio MBW / PWB is determined by Ic computed for the period -18 to -20 relative to the harvest date (figure 3). All the data are below the 1 : 1 line. So we assume $MBW = PWB * \min(1, Ic_{18})$

For each month the reproductive demand (RD) is

$$RD_{month} = NB_{month} * MBW_{month}$$

The assimilate supply for reproduction (RAS) is photosynthesis (PN) minus $VDMP$ and respiration (growth gR and maintenance mR)

$$RAS_{month} = PN_{month} - VDMP_{month} - gR_{month} - mR_{month}$$

$$\text{If } RAS_{month} > RD_{month} \text{ then } Y_{month} = RD_{month} \text{ else } Y_{month} = RAS_{month} + (RD_{month} - RAS_{month}) * K$$

Y_{month} is the bunch dry weight, which can also be output as fresh weight (including water). K is a coefficient (< 1) of reallocation of stored carbohydrates.

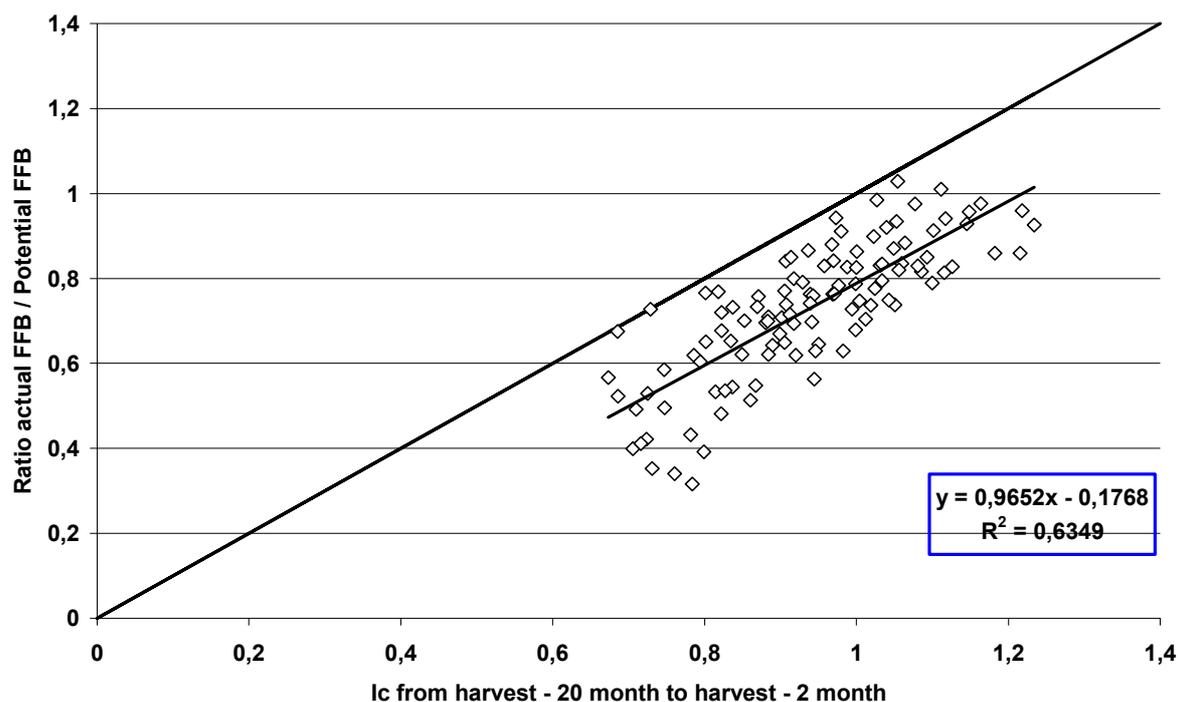


Figure 3- Relation between I_c computed from harvest – 20 till harvest – 2 months and the ratio between actual and potential FFB. The ratio never exceed 1:1 line

Calibration method

The calibration of ECOPALM model was implemented in several steps:

- *Duration from leaf (phytomer) initiation to maturity of the corresponding bunch.* We estimated this thermal duration heuristically by running the model without photoperiod effect for a series of years while testing stepwise different durations (sensitivity analysis; range tested was 20 to 60 months). The duration parameters providing the best fit of simulated vs. observed bunch harvest events gave a 49-month, total development period per phytomer. This approach is only possible if the experimental data show sufficient intra- and inter-annual variation, which was the case for Ivory Coast. This calibration process can be conducted manually (as described) or by statistical optimization implemented in conjunction with model runs.
- *Parameters governing the different development periods of the phytomer.* The ECOPALM, programmed in Delphi language, can call upon the software R (Freeware developed by R development core team) for optimization of the 4 phenological parameters $STTbPSP$, $STTaPSP$, $PPcrit$, and $PPseuil$ used to compute PNB by minimizing a cost function (RMSE) between simulated PNB and actual NB . The optimization module is *rgenoud* (genetic optimization using derivatives). The constraints are: (a) $PNB > NB$, (b) $1000 < STTaPSP < 2000$ (the PSP ends by the flowering) (c) $10000 < STTbPSP < 20000$ (d) $10.5 < PPcrit < 12.5$ (e) $30 < PPseuil < 500$.
- The same procedure is used to optimize the coefficients a and b of ISR , and $Ictph$ of the three other sensitivity stages. The optimization minimizes the RMSE between the simulated NB and the observed NB . The dates of the sensitive phases, in number of months before harvest, are parameters (from *MonthPhase1* to *MonthPhase4*). Unfortunately, *rgenoud* does not allow optimizing simultaneously integer and real parameters, requiring to proceed in several steps. One optimization is made for each value of *MonthPhasei*. The first set of

optimizations began with all IC_{tph} equal to 0.01 (no abortion) to calibrate *MonthPhase1*, *a* and *b* of *ISR*. The second set of optimizations was done with these 3 parameters set to their respective, optimized values. For each value of *MonthPhase_i* we observe the variation of RMSE to identify the minimum value, and its corresponding value for IC_{tph} .

Results and discussion

The best relation between harvested number of bunches and number of leaves initiated X months before is found for X = 49 months before harvest (figure 4). This value agrees with information obtained from plant dissections. There was no linear relation between leaf initiations and bunch maturations, but the results illustrated well the queuing-up phenomenon attributed to photoperiodism. From Adam *et al.* (2005) observations and from the rhythm of leaf emission observed here, we estimate that the event of leaf initiation occurs at rank -56 to -57 for this genotype (counting leaf positions backwards from youngest visible leaf; this is a common way of describing oil palm phenology). However, it is impossible to define precisely the relation between rank (associated with thermal time) and time because of variable temperatures.

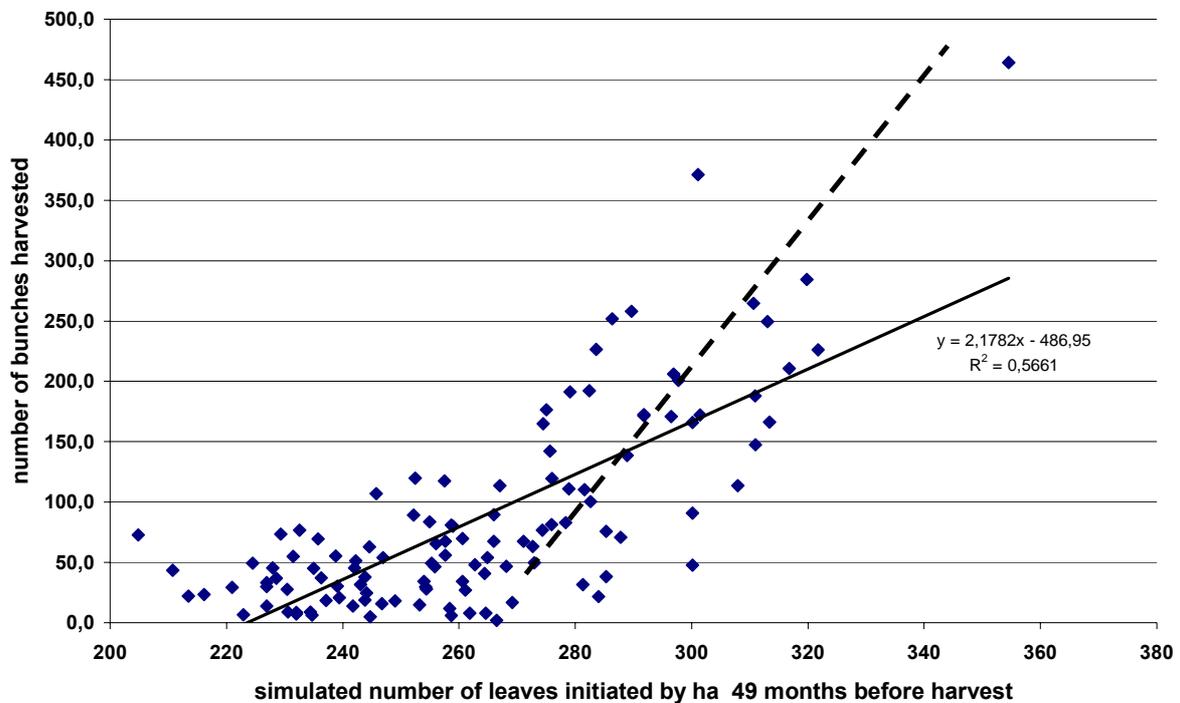
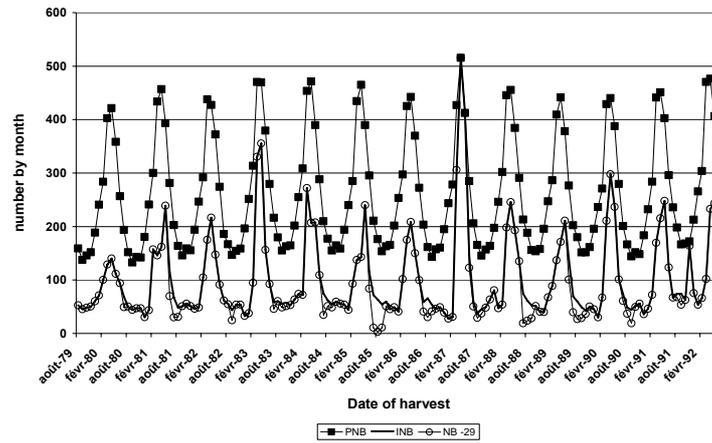


Figure 4- Relation between simulated number of leaves initiated 49 months before harvest and the actual number of bunches harvested.

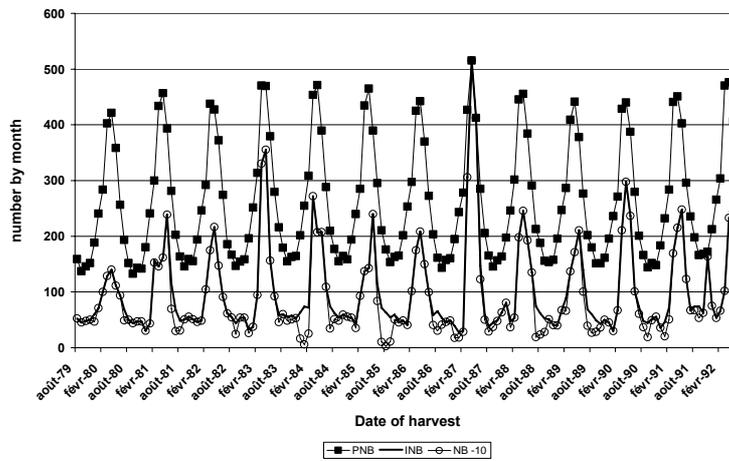
The time of initial sex initiation was found to be 47 months before harvest, 2 months after leaf (phytomer) initiation. This lag between leaf initiation and inflorescence initiation is in accordance with Corley's scheme of oil palm development (*in* Corley and Tinker 2003). The initial sex ratio is

$$ISR_{month} = \min(1, 0.08 + 0.25 * RatioIC_{month-47})$$

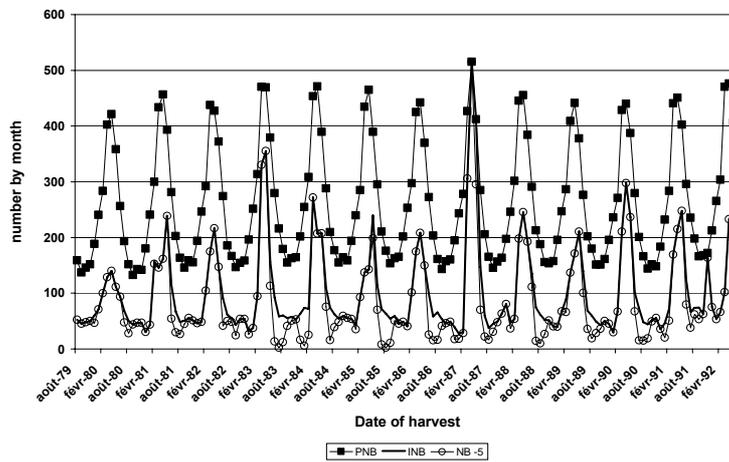
The initial number of female inflorescences is shown in figure 5. This physiological process seems to occur before any observable change in the rachis primordium. The observable change in rachis cells size begins some ranks later (Adam *et al.*, 2005).



A



B



C

Figure 5- Seasonal trend of potential number of bunches (full square), number of female inflorescences after initial sex ratio (full line), and number of inflorescences after abortion (open circles) at 29 (A) 10 (B) 5 (C) months before harvest

The three other sensitive phases provoke a reduction of female inflorescence number, or natural abortions. If values selected for *MonthPhase_i* fall into an insensitive period, the corresponding threshold $I_{c_{tphi}}$ is close to zero and RMSE increases. If *MonthPhase_i* falls into a sensitive phase, however, $I_{c_{tphi}}$ increases (ideally, to 1) and RMSE decreases. On this heuristic basis, we found that the first sensitive phase is 29 months before harvest (24 months before flowering). This occurs

between the individualization of the rachis meristem and the initiation of flower bracts (Adam *et al.*, 2005). Probably, the palm down-sizes reproductive demand at this stage under unfavorable conditions ($Ic < 1$). But it is difficult to know if this reduction of the number of female inflorescences participates in the process of differentiation of the sex, or constitutes true (but very early) abortions.

The second sensitive phase occurs 10 months before bunch maturity. This is the beginning of rapid, pre-fertilization growth of inflorescences. The third sensitive phase is 5 months before harvest (10 days after fertilization). During these two phases there is a rapid division of cells associated with pre-dimensioning of the reproductive sink, both at bunch and fruit level. Plastics sinks such as inflorescences are known to be very sensitive to internal competition during pre-dimensioning phases.

This simple model gave a good estimation of bunches number at harvest (figure 6) and of FFB production (figure 7). A better fit could probably not be expected because of model simplifications, uncertainty on some of the hypotheses used, and errors in the observed data. There is no biological justification for associating sensitive phases with single calendar months, as we did in earlier model versions. Any given month may be near the maximum of sensitivity but the sensitive phase could be longer or shorter than one month. It can also not be excluded that there are other sensitive phases during the four years of inflorescence and bunch development, which escaped identification because Ic is never attained extremely low values in Ivory Coast. Other processes determining yield may have escaped us because we worked on only one genotype which might express different patterns of sensitivity than others. But the model helped understand phenological timing the impact of environment (climate) on yield formation processes.

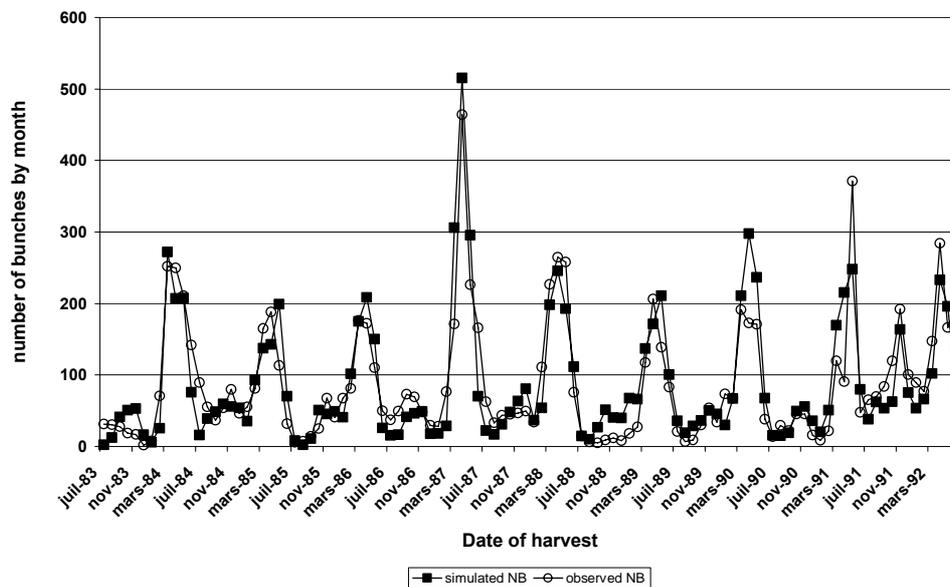


Figure 6- Simulated (full square) and observed (open circle) number of bunches per ha at harvest

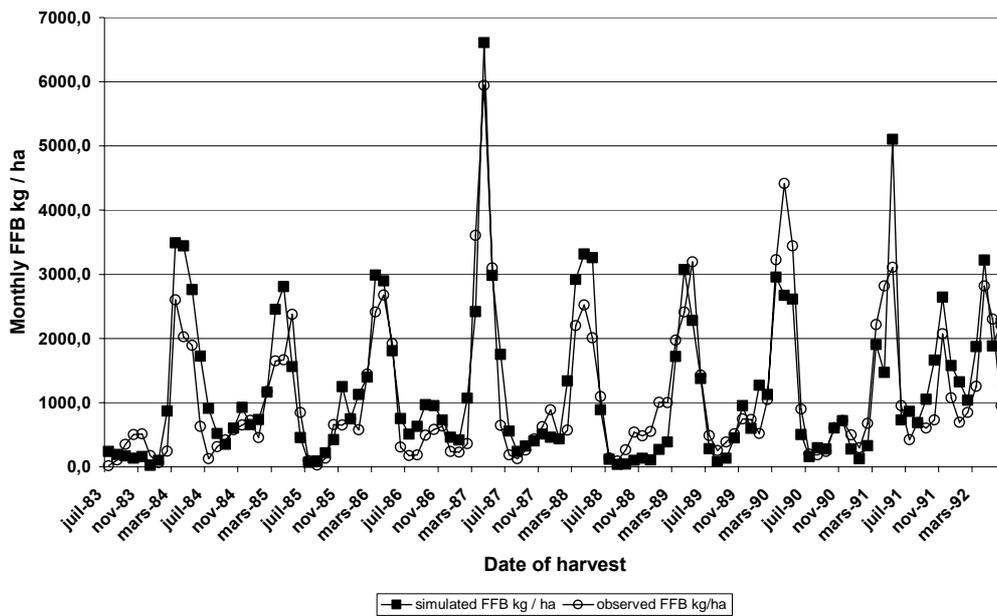


Figure 7- Simulated (full square) and observed (open circle) fresh fruits bunches production in kg per ha per month

The model can easily be adapted to other genotypes and environments having different intra-annual (seasonal) patterns of production. In fact, seasonal peaks of production vary among genotypes (unpublished data). The increase of *PPcrit* from 10 to 12 hours moves the peak of production from June to February. Of course, the variation of *STTaPSP* (duration from anthesis to harvest) would also change the date of peaks. The variation of *PPseuil* does not change the timing of peaks but increases the amplitude of variation. Consequently, this model, although far from validated for all its underlying hypotheses, can simulate the environment dependent patterns of production for contrasting genotypes.

The model also helps explaining and predicting impact of latitude. Near the equator, peaks are attenuated and they increase with latitude. During or after drought periods, such as those occurring during El Nino years in SE Asia (data not presented), production peaks can be crushed (due to low *Ic*), followed by compensatory maxima later on. In the southern hemisphere the peaks are simulated for October instead of April in northern hemisphere. Unfortunately we have very few data for the southern hemisphere.

Many questions remain open on the photoperiod sensitivity of oil palm. For obvious technical reasons, the phenomenon itself has never been reproduced experimentally. Furthermore, we are not sure whether the PSP should occur right before flowering, as simulated by ECOPALM. In cereals, floral induction happens about one month before flowering. In fact, it does not matter whether we simulate the PSP immediately before flowering or 12 or 24 months earlier because day length is not influenced by weather. If PSP would occur 24 months earlier than simulated, it would coincide with the initiation of floral bracts, coinciding with the second sensitive phase of sink adjustment.

Conclusion

This simple model confirmed the initial physiological assumptions and gave good simulations of observed data, which was our foremost objective. Validations on other locations, in particular in Indonesia, are in progress. Access to experimental and production data, as well as associated historical weather records, are a major problem. It is in the nature of a perennial crop having long physiological lags that modelling requires vast sequences of observations. Further research will

validate the model in various environments and extend it to complete crop cycles from planting to age-induced decline of production.

References

- Adam H., Jouanic S., Escoute J., Duval Y., Verdeil J.L., Tregear J.W. 2005- Reproductive developmental complexity in the African oil palm. *American Journal of Botany*. Vol 92, p 1836-1852.
- Corley R.H.V. and Tinker P.B. 2003- *The Oil Palm*. Fourth edition. Blackwell Publishing.
- Dingkuhn M., Baron C., Bonnal V., Maraux F., Sarr B., Sultan B., Clopes A., Forest F. 2003- Decision support tools for rainfed crops in the Sahel at the plot and regional scales. In *Decision support tools for smallholder agriculture in sub-Saharan Africa – A practical guide*. (Eds TE Struif Bontekes, MCS Wopereis) p. 127-139
- Dingkuhn, M., 2006- Is plant growth driven by sink regulation? Implications for crop models, phenotyping approaches and ideotypes. In: *Gene-Plant-Crop Relations. Wageningen UR FRONTIS Series, Springer* (in press).
- Dufour O., Frere J.L., Caliman J.P., Hornus P. 1988- Description of a simplified method of production forecasting in oil palm plantations based on climatology. *Oleagineux*, vol. 43, n° 7, p. 271-282
- Dufrêne E. 1989- *Photosynthèse, consommation en eau et modélisation de la production chez le palmier à huile*. Doctoral Thesis. Univ. Paris Sud. Orsay. 156 p.
- Henson I.E., 1997- Analysis of oil palm productivity. I. The estimation of seasonal trends in bunch dry matter production. *Elaeis* vol. 9, n° 2, p. 69-77
- Henson I.E., 1999- Notes on oil palm productivity. V. Evaluation of alternative mechanisms for supporting seasonal variation in dry matter production. *Journal of Oil Palm Research* vol. 11, n° 1, p. 41-52
- Henson I.E., 2004- Seasonal variation in oil palm fruit bunch production: its origins and extent. *Planter* 80 (937) p. 201-212
- Van Kraalingen D.W.G. 1985- *Simulation of oil palm growth and yield*. Doctoral Thesis, Dept. of Theoretical Production Ecology, Agricultural University, Wageningen, 106p
- Van Kraalingen D.W.G, Breure C.J., Spitters C.J.T. 1989- Simulation of oil palm growth and yield. *Agricultural and Forest Meteorology*, vol. 46, p. 227-244
- Van Keulen H., Penning de Vries F.W.T., Dress E.M. 1982- A summary model for plant growth. In : Penning de Vries F.W.T and Van Laar H.H. (editors) *Simulation of Plant Growth and Crop Production*. Simulation Monographs, Pudoc, Wageningen, p 87-94.
- Luquet D., Dingkuhn M., Kim H.K., Tambour L., Clement-Vidal A. 2006- EcoMeristem, a model of morphogenesis and competition among sinks in rice. 1. Concept, validation and sensitivity analysis. *Functional Plant Biology*, vol 33, p. 309-323.
- Mialet Serra I, Clément-Vidal A, Sonderegger N, Rounsard O, Jourdan C, Labouisse JP and Dingkuhn M, 2005- Assimilate storage in vegetative organs and coconut (*Cocos nucifera*). *Experimental Agriculture* 41 : 161-174.
- Ong, H.T. 1981- System approach to the climatology of oil palm. I Identification of rainfall and dry spells aspects *Oleagineux*, vol. 37, n° 3, p. 93-105

Ong, H.T. 1982- System approach to the climatology of oil palm. II Identification of temperature and sunshine aspects *Oleagineux*, vol. 37, n° 10, p. 443-453

Ong, H.T. 1983- System approach to the climatology of oil palm. III Interactions of fruit bun loads on development with climate *Oleagineux*, vol. 38, n° 8-9, p.469-473

Sultan B, Baron C, Dingkuhn M, Sarr B, Janicot S, 2005- Agricultural impacts of large-scale variability of the West African monsoon. *Agricultural and Forest Meteorology* **128**, 93-110.



***EcoMeristem*, a model of morphogenesis and competition among sinks in rice. 1. Concept, validation and sensitivity analysis**

Delphine Luquet^{A,B}, Michael Dingkuhn^A, HaeKoo Kim^A, Ludovic Tambour^A
and Anne Clement-Vidal^A

^ACIRAD, Amis Department, TA40/01 Av. Agropolis, 34398 Montpellier Cedex 5, France.

^BCorresponding author. Email: delphine.luquet@cirad.fr

Abstract. Because of rapid advances in functional genomics there is an increasing demand for models simulating complex traits, such as the physiological and environmental controls of plant morphology. This paper describes, validates and explores the behaviour of the structural–functional model *EcoMeristem*, developed for cereals in the context of the Generation Challenge Program (GCP; CGIAR). *EcoMeristem* constructs the plant on the basis of an organogenetic body plan, driven by intrinsic (genetic) behavioural norms of meristems. These norms consist of phenological–topological rules for organ initiation and pre-dimensioning (sink creation) and rules enabling feedbacks of the plant's resource status on the organogenetic processes. Plant resource status is expressed by a state variable called Internal Competition Index (*Ic*) calculated daily as the ratio of assimilate source (supply) over the sum of active sinks (demand). *Ic* constitutes an internal signal analogous to sugar signalling. *Ic* affects potential phytomer size, tiller initiation, leaf senescence, and carbohydrate storage and mobilisation. The model was calibrated and tested on IR64 rice grown in controlled environments, and validated with field observations for the same cultivar (Philippines). Observed distributions and dynamics of soluble sugars and starch in plant organs supported the model concepts of internal competition and the role of reserves as a buffer for *Ic* fluctuations. Model sensitivity analyses suggested that plant growth and development depend not only on assimilate supply, but also on organogenesis-based demand. If true, this conclusion has important consequences for crop improvement strategies.

Keywords: architecture, complex traits, meristem, modelling, organogenesis, *Oryza sativa* L., phenotypic plasticity.

Introduction

A major scientific challenge that has evolved during the past decade is how to improve crop-breeding methodologies on the basis of new molecular genetic knowledge (Dubcovsky 2004; Frey *et al.* 2004; Moreau *et al.* 2004). Molecular maps of genomes and information on gene function are increasingly becoming available for global crops such as rice. This is a field opening up new applications for crop models, both in the areas of phenotyping (measuring phenotypic traits that can be related to gene expression) and phenotype prediction (modelling the phenotypic impact of genes and alleles for variable environments). New models are thus needed to help build a bridge between emerging genomic knowledge and observable crop behaviour in the field. This study presents the crop model, *EcoMeristem*, developed in this context for

the CGIAR Generation Challenge Program (GCP 2005) for cereals using rice as a model plant.

In a previous paper, the authors discussed various types of plant models with respect to potential applications in genomics research (Dingkuhn *et al.* 2005). They concluded that such models, if they are to describe the whole plant (deemed essential for field applications) in variable environments (essential for breeding objectives), should be able to simulate phenotypic plasticity. Phenotypic plasticity of plant architecture, morphology and phenology is a result of genotype \times environment interactions ($G \times E$) (Wright and McConnaughay 2002; Luquet *et al.* 2005). It is, therefore, necessary not only to accurately predict the function of a gene or allele of interest, but also its phenotypic impact in a variable agronomic context. Conversely, where phenotyping

Abbreviations used: DAT, days after transplanting; DW, dry weight; FTSW, fraction of transpirable soil water; GCP, Generation Challenge Program; $G \times E$, genotype \times environment interaction; *Ic*, internal competition index; LAI, leaf area index; PAI, plant area index; PAR, photosynthetically active radiation; PET, potential evapotranspiration; RGR, relative growth rate; Rm, maintenance respiration; RUE, radiation-use efficiency; SDW, shoot dry weight; SLA, specific leaf area; Ta, air temperature; Tb, base temperature.

is the objective, models can be applied in reverse mode in order to predict genotypic parameters while using phenotype information as input. This heuristic approach is particularly relevant with respect to process-based traits and genotypic reaction norms that cannot be measured directly, such as adaptive responses of crop architecture and phenology (Hammer et al. 2002; Dingkuhn et al. 2005).

The present study does not aim at relating gene expression to whole-plant phenotype, an objective that would require tools that are currently unavailable. It only elaborates, as a first step, a modelling approach that integrates, in an interactive and dynamic way, development and growth processes in order to predict major feedbacks of environment on morphogenesis and plant structure. The objective is to achieve this with a minimal number of crop parameters and maximal ease of model parameterisation. Furthermore, emphasis is given to behavioural norms of the meristems, which are considered to be the tissues that drive plant development and which probably express many genes involved in adaptive plasticity (Jitla et al. 1997; Itoh et al. 1998; Kobayazi et al. 2002). With this, the authors hope to operate with model parameters that are closer to the effects of relevant genes, potentially enabling parameter *v.* gene (or parameter value *v.* allele) associations later on.

Since the number of interactions between development and growth processes is presumably very large, some strategic choices were made. This study focused on vegetative development only, although the entire life cycle including yield formation will be considered eventually. Furthermore, we will consider here only temperature and photosynthetically active radiation (PAR) and their effects on development rate, carbon assimilation, organogenesis and competition among growing organs for assimilates, while ignoring any specific effects of physiological stresses. This paper describes the *EcoMeristem* model, its calibration and validation for one rice genotype, and explores the model's behaviour. A sequel to this paper will extend the study to contrasting genotypes and a nutritional stress, phosphorus deficiency.

Materials and methods

The model

Underlying concepts

EcoMeristem is a whole-plant, deterministic, dynamic, radiation- and temperature-driven crop model. (The model also has a soil and plant water balance but these modules were not used in this study.) The specificity of the model is its capability to simulate competition for assimilates (supply) among growing organs (demand) (Fig. 1).

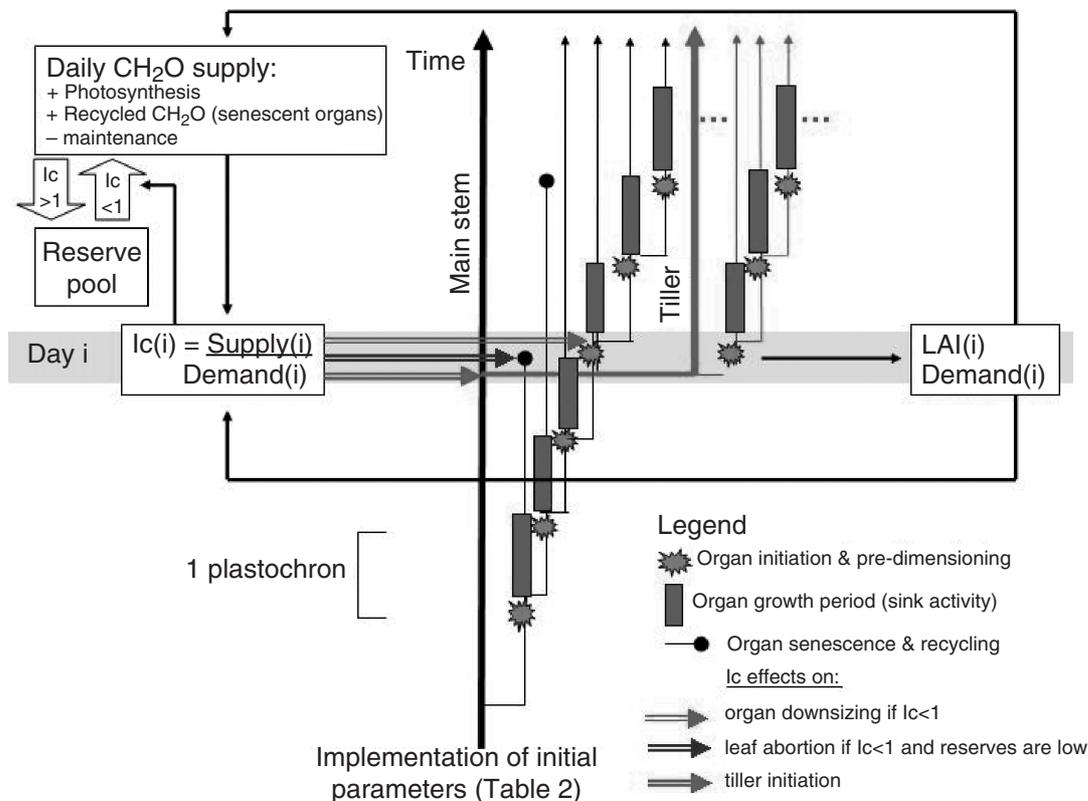


Fig. 1. Schematic diagram of *EcoMeristem* model. *Ic*(*i*), daily value of internal competition index; LAI(*i*), daily value of leaf area index, aggregate value for all existing leaves and extrapolated to field area based on plant population.

Supply is thereby simulated at the scale of the whole plant (either isolated or situated within a canopy formed by a homogenous population), whereas demand is simulated at the individual organ level, and then aggregated to provide a whole-plant demand term. This procedure allows comparison of plant level supply and demand for each time step (24 h) and to simulate feedbacks of supply / demand imbalances on organ number (organogenesis), growth rate and final size (morphogenesis). Supply/demand relationships are measured with a state variable called *Ic* (Index of internal competition; Table 1), calculated as aggregate supply divided by aggregate demand for each time step of model execution. Values of *Ic* lower than one trigger adaptive adjustments in plant organogenesis and morphogenesis, resulting in phenotypic plasticity.

Excess assimilates (when *Ic* > 1) are reversibly stored as reserves, or, if the reserve compartment is saturated, feed back on photosynthesis (product inhibition). Assimilate deficiency (when *Ic* < 1) causes two types of adaptive responses. First, the current assimilate shortfall for growth is buffered by reserve mobilisation, organ senescence (followed by recycling) and ultimately, delays in organogenetic cycles, in this order; and second, organs that are being initiated are down-sized, leading

to smaller demand when they turn into active sinks. The *Ic* conditions also branching events (in the case of grasses, tiller initiation). This system of feedbacks stabilises plant carbon balance by adjusting plant development to resources.

In contrast to assimilate supply (or source), a term that has an established physiological basis (Penning de Vries *et al.* 1989; Dingkuhn and Kropff 1996), demand for assimilates is less understood. Most agronomic crop models simply assume that incremental assimilate production (after subtraction of respiration and other losses) is reinvested in growth without limitation, and simply partitioned among organ types according to developmental stage (Penning de Vries *et al.* 1989; Sultan *et al.* 2005). This simplification cannot be upheld when we consider a dynamic body plan involving a tree structure, as well as meristems that initiate and differentiate new organs before they expand to their final size (Cookson *et al.* 2005). The plant, therefore, continuously makes commitments to new sinks, constituting demand functions that need to be adjusted to resources. A well-known example is the resource-dependent size of rice panicles (Hasegawa *et al.* 1994; Kropff *et al.* 1994; Yoshida *et al.* 2006), maize cobs (Andrade *et al.* 1999; Gambin *et al.* 2004) and wheat ears (Reynolds *et al.* 2004, 2005),

Table 1. Description of *EcoMeristem* parameters, method of calibration and estimated values for IR64 rice

Some of this information, differing slightly because an earlier version of the model was used, has been published previously in a conceptual paper on various modelling approaches (Dingkuhn *et al.* 2005; with the kind permission of the *Australian Journal of Agricultural Research*)

| Parameter Name | Definition | Unit | Method of calibration | Value for IR64 | |
|---|--|-----------------------------------|---------------------------------------|----------------|--------|
| | | | | Mean | SE |
| Parameters setting seed, seedling and population properties | | | | | |
| <i>SdDW</i> | Seed DW | mg | Measurement | 28 | – |
| <i>LDWini</i> | 1st leaf blade DW | mg | Measurement | 4.0 | 0.3 |
| <i>RSRini</i> | Root / shoot DW ratio at 1st leaf stage | – | Measurement | 1.0 | – |
| <i>SLAini</i> | 1st leaf specific leaf area | m ² g ⁻¹ | Measurement | 0.047 | – |
| <i>Pdens</i> | Plant population | m ⁻² | – | 60 | – |
| Parameters governing carbon acquisition and growth | | | | | |
| <i>RUEpot</i> | Potential radiation use efficiency (before implementation of Rm and drought effects) | g m ⁻² J ⁻¹ | Optimisation | 2.88 | 0.17 |
| <i>Krm</i> | Coefficient for the calculation of daily maintenance respiration (Rm; g glucose per g DW) using Q10 rule. Rm = <i>Krm</i> × DW at 25°C reference temperature | g g ⁻¹ | Penning de Vries <i>et al.</i> (1989) | 0.015 | – |
| <i>RESseed</i> | Fraction of seed DW mobilised ^A | – | Asch <i>et al.</i> (1999) | 0.45 | – |
| <i>Tb</i> | Base temperature | °C | Dingkuhn and Miezán (1995) | 9.55 | – |
| <i>Kdf</i> | PAR extinction coefficient ^B | – | Dingkuhn <i>et al.</i> (1999) | 0.65 | – |
| <i>STORmax</i> | Upper limit of assimilate storage in green tissues (leaf blades and sheaths) | g g ⁻¹ | Samonte <i>et al.</i> (2001) | 0.3 | – |
| Allometric parameters | | | | | |
| <i>BSR</i> | Leaf blade / sheath DW ratio | – | Measured | 0.55 | – |
| <i>RSRdem</i> | Root / shoot assimilate demand ratio | – | Optimisation | 0.310 | 0.011 |
| <i>SLAp</i> | SLA decrease for successive leaf ranks | – | Measured | 0.006 | 0.0003 |
| <i>Kls</i> | Leaf shape index (area / L × W) | – | Tivet <i>et al.</i> (2001) | 0.725 | – |
| Parameters governing organogenesis | | | | | |
| <i>PLAS</i> | Plastochron | °Cd | Optimisation | 47.3 | 1.4 |
| <i>MGR</i> | Potential meristem Growth rate | <i>PLAS</i> ⁻¹ | Optimisation | 1.60 | 0.08 |
| <i>Ict</i> | <i>Ic</i> threshold for tillering | – | Optimisation | 1.00 | 0.09 |

^AAsch *et al.* (1999) reported seed lost 75% of initial DW during germination, of which 60% reappeared in the seedling. The product of the two fractions is 0.45.

^BDingkuhn *et al.* (1999) reported values between 0.45 and 0.65 for *Kdf*. The higher value was chosen here because leaves were comparatively lax under phytotron conditions.

determined long before these sinks become active. *EcoMeristem* applies this concept to all organs of the plant except the root system (studies on root system morphogenesis are in progress to permit its detailed simulation as well).

The model assumption that a rice phytomer (entity consisting of leaf, sheath, tiller bud and internode) undergoes a dimensioning process before attaining its final size (in *EcoMeristem* a function of apical meristem size and current carbon resources) is somewhat intuitive, although it is known that (i) both meristem size (Itoh *et al.* 2005) and leaf size (Tivet *et al.* 2001) increase for subsequently formed phytomers, and (ii) leaf size of rice is strongly affected by resources such as nitrogen (Verma *et al.* 2004; IRRRI 2005), presumably through its effects on assimilate availability. Cookson *et al.* (2005) confirm for *Arabidopsis* that leaf size is, in fact, determined at an early stage of leaf development. The exact developmental period during which final leaf size is sensitive to resources is currently under study and for lack of detailed information, we assume here that the dimensioning process happens between leaf initiation and appearance.

Functional components

The main functional components of the model are: (i) assimilate production (supply function), (ii) implementation of a body plan (generation of demand functions) and (iii) arbitration between supply and demand functions (physiological feedbacks).

(i) Assimilate production

For carbon supply, the *EcoMeristem* version used here implements modules of the simple crop model SARRA-H (Dingkuhn *et al.* 2003; Sultan *et al.* 2005), which assumes that plants are part of a homogenous population having a canopy with random leaf distribution. To descend from population to plant scale, the soil surface area attributed to a single member of the population is used as basis for computations. Also adopted from SARRA-H was the simulation of an initial carbon reserve pool whose size depends on grain dry weight (DW) (parameter *SdDW*, Table 1) and the mobilisable fraction thereof (*RESseed*). Daily assimilate production and the initial seed reserves (which gradually disappears after germination) form a common pool available to all organs.

Plant area index PAI [the single-plant equivalent of leaf area index (LAI)] is computed from green-leaf dry weight by applying an empirical, allometric rule for blade/sheath DW ratio (Luquet *et al.* 2005; Table 1) and the specific leaf area (SLA, $\text{m}^2 \text{g}^{-1}$) attributed to leaf blades according to their position n on the stem. SLA is a steadily decreasing function of leaf rank n (Luquet *et al.* 2005) computed here with two parameters: *SLAini* and *SLAp* (Table 1):

$$\text{SLA} = \text{SLA}_{\text{ini}} - \text{SLA}_{\text{p}} \times \ln(n) \quad (1)$$

This equation reproduces the development-stage-dependent decrease of SLA observed in rice (Asch *et al.* 1999) and generally in cereals, using leaf rank as measure of development stage. For model output, a distinction is made between structural SLA [as computed with Eqn (1)] and actual SLA, which includes simulated transitory carbon reserves, considered to be equally distributed among all green leaf sheaths and blades. For this reason, observed data used for model calibration should be based on measurements made in the morning, when transitory reserves are smallest. No structural effects on temperature or radiation levels on SLA are taken into account.

Lambert–Beer's law of logarithmic light quenching (Verhoef 1985) is applied to LAI to compute PAR interception using an extinction coefficient *Kdf* (Table 1). Then carbohydrate assimilation is computed by multiplying intercepted PAR with a radiation-use efficiency (RUE) parameter. Contrary to common definitions of RUE (Monteith 1994; Kiniry *et al.* 2001), this parameter is calibrated so as to include root

growth and maintenance respiration, which is subsequently calculated and subtracted from the assimilation term (this provision was made because RUE is known to decrease in the presence of a large biomass due to maintenance respiration; Penning de Vries *et al.* 1989). The model provides for drought stress effects on assimilation (function of fraction of transpirable soil water, FTSW), but this was not used in this study.

Maintenance respiration (R_m) was considered to be proportional to shoot DW (SDW) and a power function of air temperature (T_a) according to the $Q_{10} = 2$ rule (doubling of rate for every increase of T_a by 10°C), according to Penning de Vries *et al.* (1989).

(ii) Implementation of a body plan

Developmental processes were implemented along thermal time, starting with germination (1st-leaf stage). The thermal time elapsing in 1 d was defined as the difference between the mean daily air temperature and the base temperature T_b (Table 1). Organ initiation (new leaves and tillers) was implemented with a genotypic plastochron (Table 1) which spanned several days and was statistically optimised against a target file containing morphological observations (Table 2).

The topology of the plant consists of a principal axis or main stem, constituted by a sequence of phytomers (Fig. 1). Each phytomer consists of a leaf (blade and sheath), a virtual axillary node and an internode (internodes were not attributed mass and dimensions in this study because rice plants remained vegetative). An open-ended number of tillers can be created, depending on an evolving number of potential sites (one bud per phytomer on main stem and tillers), but their actual number depends on assimilate availability and genotypic sensitivity to it (this will be explained further below). Each tiller is defined by its time of initiation and the leaf on the main stem with which its first leaf will be synchronous, according to principle of cohorts (Hanada 1993; Tivet *et al.* 2001). All subsequent leaves produced on the tiller, as well as internode elongation and panicle growth (not simulated in this study), are from then on synchronised with the main stem.

The expansion of a new leaf to its final size happens during a single phyllochron after initiation of the corresponding phytomer. Carbon demand of an expanding leaf is thus considered only once its tip emerges from the enclosing sheath of the previous leaf (i.e. when its sink strength becomes significant), and subsides when the next leaf appears.

This is a major simplification because in fact, the periods of expansion of successively appearing leaves overlap to some extent in rice. Two or three leaves queue up in the tube formed by several sheaths, and leaf initiation therefore happens earlier than simulated by the model (Jitla *et al.* 1997; Itoh *et al.* 1998; Miyoshi *et al.* 2004). As in all grasses, the development and growth events on shoot axes of rice are highly coordinated (Fournier *et al.* 2005). At leaf tip appearance, the ligule of the same leaf differentiates at its junction with the sheath (collar), which is at that time hidden in the previous leaves' sheaths. Once the collar of emerges from the enclosing sheath, the elongation of the leaf blade ends (Williams 1975; Skinner and Nelson 1995), probably involving long-distance signalling (evidence summarised by Fournier *et al.* 2005). There are major differences among grass species regarding the number of successive phytomers whose development overlaps in time. In *Poa pratensis* L., a new leaf is initiated only once the previous leaf is nearly fully expanded, whereas in maize five leaves having different development stages grow at the same time (Sylvester *et al.* 2001). Rice is intermediate, with a total of three leaves developing at the same time (Sylvester *et al.* 2001). Their development is coordinated such that the appearance of the tip of leaf n coincides with the ligule emergence (and thus, the end of rapid elongation) of leaf $n - 1$. Consequently, only one visible leaf per culm is undergoing rapid (linear) elongation at any given time, while two other leaves hidden in the sheath elongate much more slowly (exponential elongation phase).

Since little evidence of this specific behaviour of rice can be found in referenced journals, we present here an example of elongation kinetics

Table 2. *EcoMeristem* model input variables, output variables and measured variables used for statistical parameter optimisation

| Variable | Physical scale | Temporal scale | Unit |
|--|---|----------------|---|
| Model input variables ^A | | | |
| Mean air temperature Ta | – | daily | °C |
| PAR | – | daily | MJ m ⁻² d ⁻¹ |
| Model output variables (available for each time step) | | | |
| Organ dry weight | Leaf blades, sheaths, root system | daily | g plant ⁻¹ |
| Leaf area | Per leaf, tiller or plant | daily | m ² |
| Leaf and tiller number | Whole plant | daily | – |
| Senescent leaf number | Whole plant | daily | – |
| Organ length | Individual leaf, total shoot (plant height) | daily | m |
| Specific leaf area (SLA) | Per leaf blade or plant | daily | m ² g ⁻¹ |
| Leaf growth rates | Individual leaf | daily | mm d ⁻¹ , mm ² d ⁻¹ , mg d ⁻¹ |
| Carbon reserve pool | Plant | daily | g plant ⁻¹ , mg g ⁻¹ |
| Index of competition (Ic) | Plant | daily | – |
| Variables measured for parameter optimisation (target file for this study) | | | |
| Leaf number | Main stem | 36 DAT | g |
| Leaf number | Whole plant | 36 DAT | – |
| Leaf blade length and dry weight | Last fully expanded leaf on main stem | 36 DAT | m, g |
| Tiller number | Whole plant | 36 DAT | – |
| Shoot dry weight | Whole plant | 36 DAT | g |

^AInput variables for soil moisture and atmospheric demand are not provided because water balance was not simulated in this study.

of subsequently appearing leaves on IR64 rice (Fig. 2: observations on one plant taken from the experiment described below). Therefore, the simulation of leaf growth confined to the duration of a single phyllochron, as done here in *EcoMeristem*, is a simplification because it ignores the slow (exponential) growth of leaves before their appearance, but this probably causes only a small bias with respect to the timing of carbon demand in expanding leaves.

On the main stem, potential leaf size increases from one phytomer to the next (Tivet *et al.* 2001), a trend that is associated with an increase of the size of the apical meristem (Itoh *et al.* 1998, 2005; Asai *et al.* 2002). In *EcoMeristem*, the apical meristem grows during each plastochron by a constant factor (parameter Meristem Growth Rate, *MGR*; Table 1) if assimilate supply is non-limiting. It grows less if $Ic < 1$. The potential DW of a new leaf is assumed to be proportional to the meristem size at its appearance. Therefore, potential DW of leaf n on the main stem is equal to final, structural DW of leaf $n - 1$, multiplied by *MGR*. Final DW of leaf n is equal to its potential DW, or smaller if $Ic < 1$. The down-sizing of the leaf when $Ic < 1$ is non-linear (using Ic^{-2} as factor, instead of Ic) because a linear function was found to have unrealistic, disruptive effects on the simulation process. In summary the final DW of a new leaf on the main stem depends on that of its predecessor, the genotypic value of *MGR* and the resource situation (value of Ic) at the time of its appearance.

Leaves produced by tillers are initially smaller than other leaves of the same cohort, but leaves appearing subsequently catch up in size with those on the main stem (Tivet *et al.* 2001). In the model, we assume that the first leaf produced by a tiller has an intermediate (mean) size between the leaf simultaneously produced on the main stem (same cohort), and the very first leaf produced on the main stem. Subsequent leaves produced by the tiller are pre-dimensioned at the time of their initiation as the mean weight of the previous leaf on the main stem and that on the concerned tiller, multiplied by *MGR*. Consequently, the weight of leaves appearing on tillers asymptotically converges towards

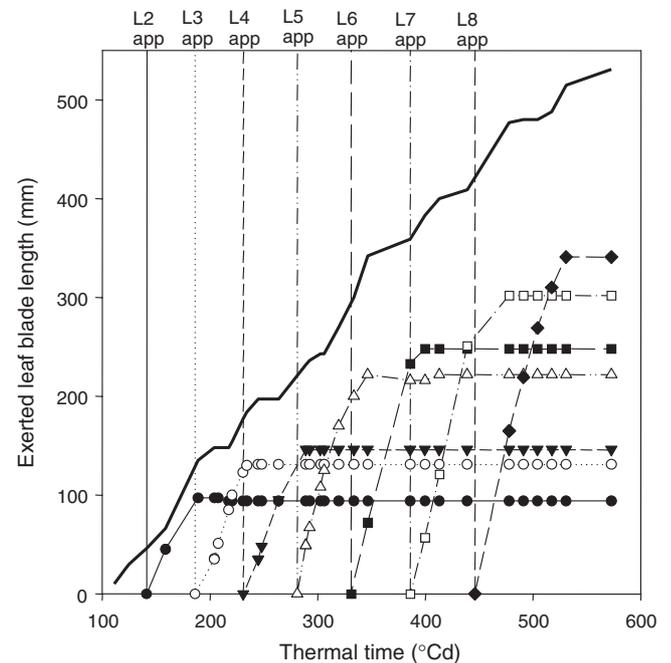


Fig. 2. Kinetics of the elongation of seven successive leaf blades on the main culm of IR64 rice (L2–L8, visible parts only), from tip appearance (app) to constant length. The continuous, bold line indicates plant height from shoot base to the tip of the youngest, fully expanded leaf. Kinetics of aggregate sheath length can be estimated from the difference between plant height and the length of the longest leaf present on the culm. Only one of four replications is shown.

that of leaves appearing on the main stem, and leaf size on older tillers (having several phytomers) is similar to that on the main stem.

The root system is not simulated with the same amount of detail as the shoot, although a detailed version is being developed. The present version of *EcoMeristem* considers the root system as a bulk compartment of the plant, with a daily carbon demand that is equal to the total plant carbon demand simulated on the previous day, multiplied with a genotypic parameter *RSRdem* (Table 1).

Contrary to some other architectural models (e.g. GREENLAB; Yan *et al.* 2004), organ lifespan is not forced by *EcoMeristem*. Senescence is triggered by assimilate shortage, resulting in 'recycling' of the oldest leaves and youngest tillers on the plant. Leaf longevity is known to depend also on nitrogen supply (Dingkuhn *et al.* 1992), which is not simulated at present. However, the feedbacks of assimilate shortage on leaf size and mortality implemented here are bound to occur as well when RUE is reduced by N deficiency, or any other physiological stress for that matter. Future modules for mineral and other stresses can thus make use of the existing mechanism for senescence, provided that their effect resembles that of assimilate starvation.

(iii) Arbitration between supply and demand functions

At the core of this modelling concept is the hypothesis that plant growth is not only supply driven [which is the case for most agronomic models such as APSIM (Wang *et al.* 2002), STICS (Brisson *et al.* 1998 or DSSAT (Jones *et al.* 2003), but also demand driven. The underlying assumption is that organ development begins with cell divisions (determining potential size and thus, sink capacity) and ends with expansion (during which demand for resources is greatest). Although cell division and expansion phases overlap (Tardieu *et al.* 2000), there is reason to assume that meristem activity must be regulated through supply-related feedbacks in order to efficiently adjust organ size to fluctuating resources (Luquet *et al.* 2005; Murchie *et al.* 2005). In fact, recent findings on sugar signals regulating meristem activity support this concept at the molecular scale (Sherson *et al.* 2003; Heyer *et al.* 2004).

In *EcoMeristem*, the ratio between aggregate carbon supply and demand at the whole-plant scale (state variable *Ic*) serves as a signal influencing development processes. In order to keep the model reasonably simple and transparent, *Ic* directly affects only two processes deemed crucial for adaptive responses of morphogenetic processes: down-sizing of new organs at the time of their initiation if $Ic < 1$ and enabling of tiller production if $Ic > Ict$, with *Ict* being a threshold parameter potentially smaller or larger than 1.

These two effects of *Ic* are strategic in the sense that they do not alleviate assimilate shortfalls immediately, but during subsequent plastochrons when they have an impact on sink activity (growth of the organs initiated). There are, however, also immediate effects of supply and demand imbalances, required to keep the carbon balance intact.

Case of $Ic > 1$:

- Storage of excess assimilates in vegetative tissues (leaf blades, sheaths and internodes, once simulated).
- Proportional reduction of photosynthesis if storage reaches its physiological limits (parameter *STORmax*).

Case of $Ic < 1$:

- Mobilisation of stored assimilates.
- Senescence of the oldest leaf if reserve mobilisation is insufficient to satisfy demand (senescence of the youngest tillers — if leaf recycling is insufficient — is not yet implemented in the current version of the model).
- Delay of organ expansion and extension of current plastochron if the above are insufficient.

These processes, generally known but insufficiently studied to model them quantitatively, were programmed rather intuitively. They are necessary, however, to account for the fact that plant development is not only based on organogenesis but also on organ death and recycling of internal resources.

Model parameters and input/output variables

When applied to non-water limited environments, the *EcoMeristem* model uses 18 crop parameters (Table 1). Preliminary, unpublished observations indicated that most of these parameters vary little among rice cultivars. Strong genotypic variation, and thus the need for careful parameterisation, was found in seed DW (*SdDW*, a parameter necessary for the calculation of the initial pool of carbon reserves), first-leaf DW (*LDWint*), plastochron (*PLAS*), meristem growth rate (*MGR*) and the critical *Ic* value for tillering (*Ict*). The first two parameters can be easily observed on seeds and seedlings in the course of germination tests, but the last three parameters calibrate organogenetic responses and thus, are quite inaccessible to measurement.

For applications that do not consider water deficit or photoperiodism, the model uses only two weather input variables: mean daily air temperature and PAR (Table 2). When applied to water-limited environments and photoperiod-sensitive genotypes, additional input variables such as potential evapotranspiration (PET), soil depth and water holding capacity, rainfall/irrigation and geographic latitude are needed.

The model provides a large number of output variables at daily time steps, describing plant morphology (structural and total DW, dimensions and surface area of organs), organ number and topological position, as well as *Ic* (as an indicator of assimilate status) and transitory carbohydrate reserves at the whole-plant scale (allocated to vegetative shoot organs proportionally to their structural DW, whereas roots are not considered storage organs). Only the most important output variables are listed in Table 2, whereas others that are only relevant for reproductive growth phases and water limitation are disregarded here.

Programming aspects

The current version of *EcoMeristem*, which is a prototype for research purposes, was programmed with Matlab software (version 6.5, Mathworks Inc., Natick, MA). The model is now being implemented in a third generation programming environment (Delphi, version 5, Borland-France, Paris, France) using an object approach, destined for routine phenotyping applications in the context of genetic and functional-genomics research, marker development for breeding, and plant ideotype development. The object approach permits defining generic entities (such as organ types) that can be more easily adapted to different plant topologies.

Model calibration

The model was calibrated for IR64 (*Oryza sativa* L. indica type) rice grown under the controlled, experimental conditions described later. Parameterisation, where it used experimental data, was performed separately for each of the four experimental replications, in order to obtain standard errors for parameter values. Results of parameter optimisation are summarised in Table 1.

Information on parameter values was derived from three sources (Table 1), and implemented in the following order: (1) generic information from the literature, (2) direct calibration with measured observations, and (3) indirect calibration with statistical parameter optimisation against measured observations by running the model. Parameters derived from the literature included coefficients for calculating temperature- and biomass-dependent maintenance respiration (*K_{rm}*), the DW fraction of seed available as reserves (*RESeed*), and the extinction coefficient for PAR (*K_{df}*) (literature citations in Table 1). There is considerable uncertainty on the accurate

value of these parameters and on their variability. For *Tb*, we used a value for IR64 obtained heuristically (by model fitting) from field observations (Dingkuhn and Miezan 1995). For *Krm*, a generic value proposed by Penning de Vries *et al.* (1989) was used, but since maintenance is very small during vegetative growth, the accuracy of this parameter value has little bearing on the results of this study. Growth models are very sensitive to *Kdf*, a parameter that is difficult to measure. We used a value adapted from Dingkuhn *et al.* (1999), a study that compared several methods for the estimation of *Kdf*. Also uncertain is the value of *STORmax*, partly because reserves are rarely measured in vegetative-stage plants and partly because it is difficult to estimate the upper limit of storage. We assumed here that the storage capacity of leaves and stems observed by Samonte *et al.* (2001) for leaves and sheaths of several rice cultivars at heading stage can be extended to these organs during vegetative growth stages as well.

Parameters adjusted manually with direct measurements included all initial crop parameters derived from germination tests (individual seed DW, *SdDW*; first-leaf DW, *LDWini*; first-leaf SLA, *SLAini*; and root/shoot DW ratio at first-leaf stage, *RSRini*), as well as some parameters adjusted manually on the basis of plant observations at 36 d after transplanting (DAT) (individual leaf blade/sheath DW ratio, root/shoot DW partitioning ratio and a coefficient setting the decrease in SLA for subsequently appearing leaves, eqn 1). Lastly, some less accessible parameters describing morphogenetic behaviour (*PLAS*, *MGR* and *Ict*) and *RUEpot* were optimised statistically by running the model while varying parameter values.

Optimisation was done with utilities available on the Matlab software package, which also served as programming environment. The Nelder-Mead method (Nelder and Mead 1965) was applied to a maximum of three parameters at a time. The optimisation procedure required establishing a standardised target file containing the observations in a format that corresponds to model output, in order to evaluate prediction errors. Table 2 (bottom) provides details of this target file.

Model validation

The model was field-validated with a published field experiment conducted in the Philippines in the 1988 dry season (site of Muñoz, 120°56' E, 15°45' N, altitude 48 m, mean daily PAR 11.2 MJ m⁻² d⁻¹; Schnier *et al.* 1990), using the same cultivar IR64 for which the model had previously been calibrated under controlled, growth chamber conditions (parameter values as in Table 1 except plant population, which was 180 plants m⁻² in the field and 30 plants m⁻² in controlled environments). Plant establishment method was similar in both cases (wet, direct seeding of pre-germinated seed). For the field experiment, sequential observations were available on bulk leaf blade and stem (essentially, sheath) DW, plant height, tiller number and leaf area. Since these data were based on soil surface area, values were transformed to single-plant scale. Global solar radiation data from the experiment were converted to PAR with a factor of 0.49 (Kropff and van Laar 1993). The field experiment was composed of six nitrogen input treatments between 0 and 150 kg ha⁻¹. Since *EcoMeristem* is not sensitive to N resources, its simulations were compared with all of the N treatments.

Growth chamber experiment

IR64 rice seed (*O. sativa* indica type), provided by the International Rice Research Institute (IRRI) in the Philippines and multiplied by the authors, was grown in controlled environment at CIRAD (Montpellier, France) between April and June 2004, as part of a larger experiment involving several genotypes and nutritional treatments. Only relevant information is reported here.

Seeds were germinated for 4 d at 33°C in illuminated germination chambers, and then selected for seedling uniformity. Seedlings (first-leaf stage) were then transferred to drained, 1-L pots containing fine quartz sand and watered daily with a culture solution (concentrations in mM:

KH₂PO₄ = 0.21, K₂HPO₄ = 0.06, KNO₃ = 1.98, Ca(NO₃)₂ = 2.96, MgSO₄ = 0.61, KCl = 0.1, (NH₄)₂SO₄ = 0.53, MnSO₄ = 2.9 × 10⁻³, (NH₄)₂MoO₄ = 6 × 10⁻⁵, CuSO₄ = 6.3 × 10⁻², ZnSO₄ = 2.5 × 10⁻³, H₃BO₃ = 7.4 × 10⁻³, EDTA-Fe = 0.206, pH = 5.5) to maintain field capacity. Air temperature in the culture chamber was 28°C/23°C (day/night), relative air humidity was 60%/80%, and PAR at the level of plant tops was 8 MJ m⁻² d⁻¹ supplied over a 14-h photoperiod. Light was supplied with halogen lamps at ~0.8 m from plant tops. To avoid effects of chamber heterogeneity, plants were rearranged daily. Temperature at plant base and PAR at plant tops were monitored continuously to calculate daily mean temperature and cumulative PAR. The experiment had four replications in a block design, with several pots per block to permit destructive sampling at several growth stages. After each destructive sampling, pots were rearranged to form a plant canopy at 30 plants m⁻² including single rows of border plants. All remaining plants were harvested at 36 DAT for measurements constituting the target file for model calibration (Table 2).

Measured variables and measurement schedule are summarised in Table 3. Sample size per replication was one plant. Destructive sampling was done in the morning in order to avoid DW variation caused by transitory reserve accumulation in leaf blades, which is most pronounced in the afternoon (Munns and Weir 1981; Walter and Schurr 2005). Root systems were sampled in bulk and washed thoroughly to remove sand. All samples taken for DW measurements were dried in ventilated ovens at 70°C until constant weight, and then weighed with a precision balance (resolution 0.1 mg). Samples for sugar analyses were deep frozen and processed as described in the following section. Leaf blade area was estimated from blade length and width using an allometric coefficient of 0.725 (Tivet *et al.* 2001). Specific leaf area (SLA) was calculated by dividing individual leaf blade area by the corresponding DW. Leaf appearance was defined as the time when the leaf tip emerged from the enclosing sheath. Blades were considered to have achieved their final length when the ligule had emerged from the previous leaf's sheath.

Analytical methods

Dry matter and sugar concentrations of bulk plant parts were determined after lyophilisation. Samples were ground with liquid nitrogen with a ball grinder (Mixer Mill MM 200, Retsch, Germany). Sugars were extracted

Table 3. Measurement schedule (days after transplanting, DAT) and derived variables (underlined)

| Measured and <u>estimated</u> variables | DAT |
|--|--------------------|
| Air temperature at plant base and PAR at level of plant tops | Continuous |
| Individual leaf blade and sheath DW on all stems; root DW | 12, 19, 25, 30, 36 |
| Individual specific leaf area (SLA), <u>root/shoot DW ratio</u> and <u>blade/sheath DW ratio</u> | 12, 19, 25, 30, 36 |
| Individual leaf blade and sheath size (length, width and area) | Daily |
| Leaf appearance and <u>phyllochron</u> (thermal time between appearance of 2 leaves) | Daily |
| Tiller appearance | Daily |
| Plant height (distance from ground to tip of last fully expanded leaf on the main stem) | Daily |
| Glucose, fructose, sucrose and starch concentration (bulk blades, sheaths and roots) | 12, 19, 25, 30 |

three times from 30-mg samples with 1 mL 80% ethanol for 30 min at 80°C, and then centrifuged. Soluble sugars were contained in the supernatant and starch in the sediment. The supernatant was filtered in the presence of polyvinyl polypyrrolidone and activated carbon to eliminate pigments and polyphenols. After evaporation of solute with Speedvac (RC 1022 and RCT 90, Jouan SA, Saint Herblain, France), fructose, glucose and sucrose were quantified by high performance ionic chromatography (HPIC, standard Dionex) with pulsed amperometric detection (HPAE-PAD). The sediment was solubilised with 0.02 N soda at 90°C for 2 h and then hydrolysed with α -amylglucosidase at pH 4.2 for 1.5 h. Glucose was quantified as described by Boehringer (1984) with hexokinase and glucose-6-phosphate dehydrogenase, followed by spectro-photometry of NADPH at 340 nm (spectrophotometer UV/VIS V-530, Jasco Corporation, Tokyo, Japan).

Results

Model parameter values for IR64 rice obtained in controlled environments

The model parameter values obtained for IR64 are presented in Table 1. Due to a very homogenous population and controlled culture conditions, measurement-derived parameter values were very similar among the four replications, even in the case of statistical parameter optimisation, as indicated by the standard errors of the mean (SE). The threshold parameter for tillering (*Ict*) was 1.0, indicating that IR64 did not require any assimilate surplus (relative to current demand) to initiate a tiller. The meristem growth rate (*MGR*) was 1.6, indicating that each subsequent leaf produced on the main stem was up to 60% heavier (if supply was not limiting) than its precursor. Whether the meristem actually grew in size at this rate remains to be confirmed, although non-quantitative, microscopic observations on dissected apical meristems appeared to confirm the hypothesis (results not presented). Detailed observations on meristem development are currently in progress.

Simulation of observed plants

Morphogenesis

The calibrated model accurately reproduced the observed time courses of shoot and whole-plant dry weight, as well as tiller production (Fig. 3). Furthermore, the observed distribution of dry weight and leaf area between the main stem and various tillers, and among leaf positions on the culms, was simulated accurately (Fig. 4: individual leaf area of fully expanded leaves; dry weights and physical dimensions were simulated but not presented here).

Carbon dynamics

Since the *EcoMeristem* model simulates carbon reserve dynamics in the plant and their feedbacks on development processes, we investigated the distribution among organs of soluble sugars and starch in the course of vegetative development (Fig. 5). No significant amounts of polyfructans were found. Hexose (glucose and fructose)

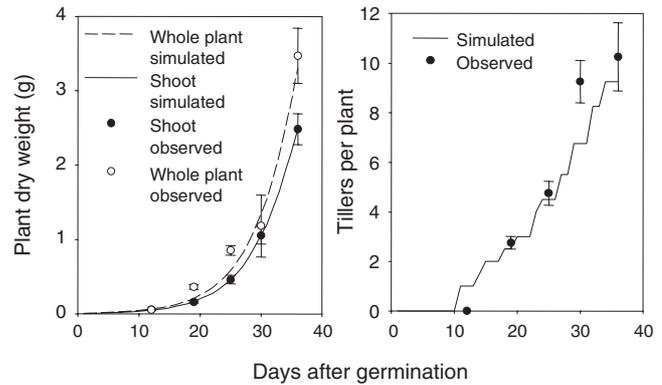


Fig. 3. Simulated and observed shoot DW and tiller number for IR64 rice in controlled environments. Vertical bars represent standard error of four replications.

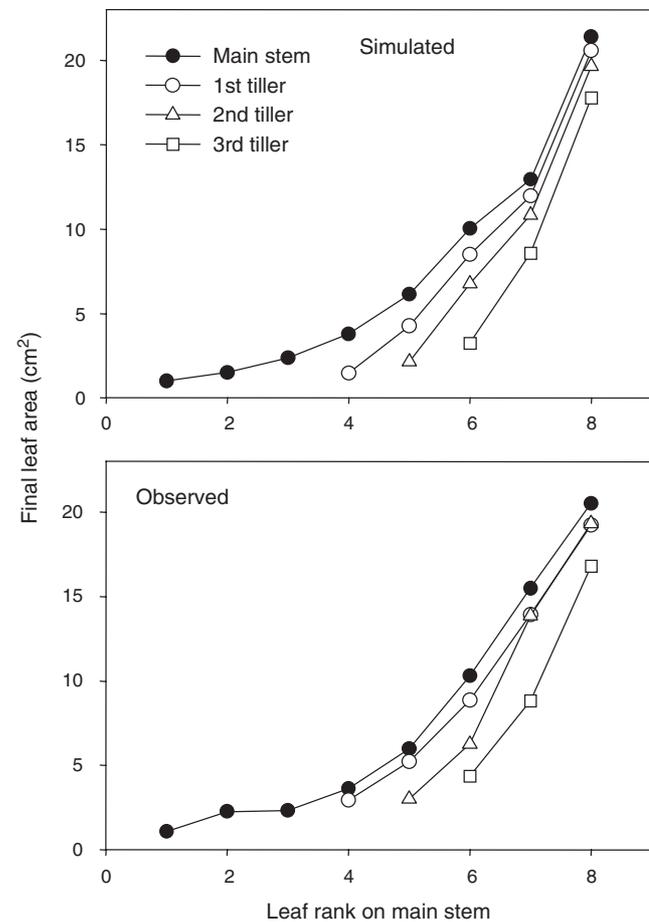


Fig. 4. Simulated and observed individual area or fully expanded (ligulated) leaf blades by chronological rank on culm and primary tillers for IR64 rice in controlled environments.

concentrations increased consistently with plant age in leaf blades and sheaths ($P < 0.05$), but not significantly in roots. Sucrose concentrations were greatest in blades, smaller in

sheaths and smallest in roots. They did not change during the period of observation in leaf blades, but decreased significantly in sheaths. Lastly, starch concentrations were greatest in sheaths and decreased significantly over time. They were intermediate in leaf blades and very small in roots, with no significant trend over time.

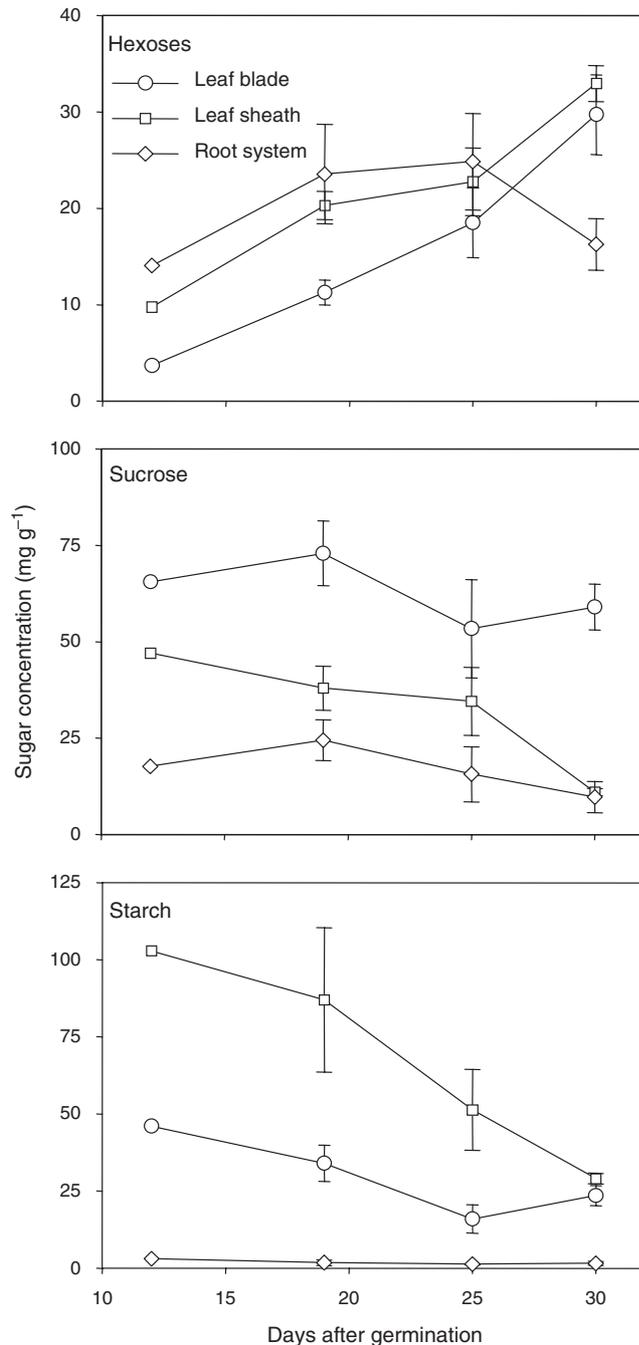


Fig. 5. Observed dynamics of sugars in bulk leaf blade, sheath and root samples taken from IR64 rice at four sampling dates. Vertical bars represent standard error of three replications.

It is difficult to relate these observations to variables simulated by the model, because the model considers only a general assimilate reserve pool in the plant without specifying substance classes and organs. In Fig. 6, observed sucrose and starch concentrations (supposed to constitute main reserve compounds) in sheaths (considered a storage organ) were compared with the model outputs I_c (which is an index of assimilate abundance) and weight fraction of reserves in the shoot. Although these variables cannot be compared in quantitative terms, the simulated and observed variables showed the same trend and appeared to be correlated (although with four points, this cannot be asserted statistically). Note that for model calibration, only morphological observations and no chemical measurements were used.

Field validation

The model as calibrated for IR64 under growth chamber conditions was validated with field data for the same cultivar published previously (Schnier *et al.* 1990). The model was run with climate data from the field site in the Philippines and the respective plant population density, which was much greater than that in the growth chamber (180 plants m⁻², as opposed to 30 plants m⁻²). None of the original crop parameters was modified.

Since the model does not consider the nitrogen status of the crop, and the field experiment consisted of six levels of N input, we compared simulations with all N treatments in the field for the initial 36 d of growth (Fig. 7). Simulated shoot

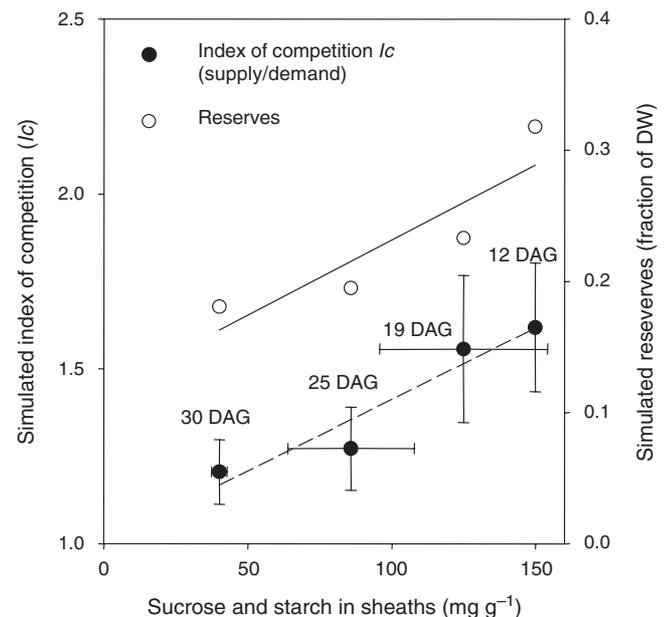


Fig. 6. Relationship of simulated carbohydrate reserves and internal competition index (I_c) v. observed reserve (starch + sucrose) concentration in leaf sheaths for IR64 rice during vegetative growth.

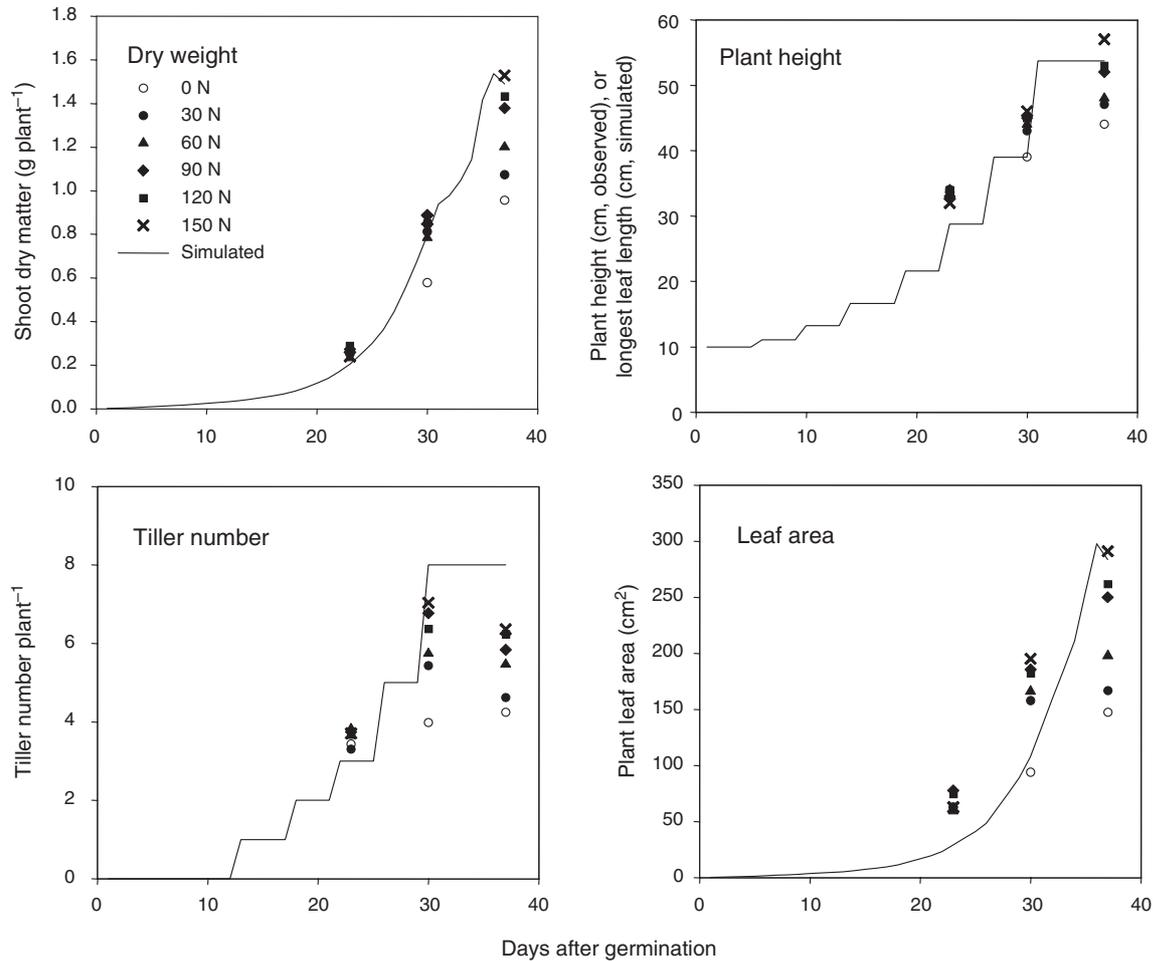


Fig. 7. Field validation of the *EcoMeristem* model, parameterised in controlled environments for IR64 rice, using growth observations for the same cultivar under irrigated conditions in the Philippines (source of data: Schnier *et al.* 1990; direct seeded at 180 plants ha⁻¹). Since the model does not simulate nitrogen effects, simulations were compared with six different levels of N application between 0 and 150 kg N ha⁻¹. Best fit is observed for the higher N levels in the field, consistent with the non-limiting N resources applied in the growth chamber.

dry weight per plant gave an excellent fit with observed values for the treatments having high N inputs, which is consistent with the fact that the model was calibrated on plants grown with non-limiting N supply. The same was observed for plant height. Predictions of tiller number were good until 30 d after germination but were followed by an over-estimation on day 36. In fact, the authors of the field data reported a slump in tillering at this stage, which was subsequently corrected with a second application of N, shortly before panicle initiation (Schnier *et al.* 1990). Consequently, the slump in tiller production was due to a temporary exhaustion of soil N supply and thus, not simulated by the model. Lastly, observed plant leaf area (calculated from LAI and population density) increased earlier than the corresponding, simulated values, but the two were similar at 36 DAT.

In summary, the model parameterised under growth chamber conditions gave a reasonably good prediction of field

observations for the same genotype. It can thus be considered acceptable for non-water- and -nitrogen-limited conditions during vegetative development.

Sensitivity analysis

The model was extensively tested for its sensitivity to input variables and crop parameters, in order to explore its behaviour and evaluate the biological coherence of its responses. We present here examples of the effect on model outputs of variations of PAR (input variable) and three crop parameters that govern organo- and morphogenesis, and thus, plant type (*MGR*, *PLAS* and *Ict*). These parameters affect not only the rate of increase of the size of consecutively appearing leaves (*MGR*), the rate of leaf appearance (*PLAS*) and the sensitivity of tillering to assimilate supply (*Ict*), but also, by way of feedback, most other morphological properties of the plant. The sensitivity analyses were limited to the initial 36 d

of plant development in order to stay within the limits of the available experimental evidence. The parameter values used as basic setting were the ones for IR64 grown under growth chamber conditions (Table 1).

(i) Effects of environment input parameters

PAR levels ranging from 5 to 20 MJ m⁻² d⁻¹ affected shoot DW approximately linearly up to 12 MJ m⁻² d⁻¹ and then did not increase dry weight any further (Fig. 8). The insensitivity of growth to higher radiation levels was not due to light saturation (which is not to be expected at this level of PAR because of the strong leaf inclination of rice; Dingkuhn *et al.* 1999) but to limited demand for assimilates. A higher value for *MGR* (enabling potentially larger leaves) or a lower *Ict* (enabling more responsive tiller production) would be necessary to provide positive growth responses to higher PAR levels, but it is not certain that the plants would indeed respond in this manner. When applied to dense crop stands in the field (where competition among plants for light is more severe than in our growth chamber experiment), the model predicts positive growth responses for the full range of naturally occurring light levels (data not presented).

It is characteristic of this model that simulated growth responses to parameters and input variables show oscillations, both in time and in response to parameter values, as observed for example for lower PAR ranges in Fig. 8. These oscillations are due to the impact of facultative development events such as initiations of tillers and leaf cohorts, which temporarily increase demand for assimilates and thus reduce the size and/or rate of appearance of leaves. This phenomenon is more pronounced at low PAR levels because of severe

competition among organs for assimilates, associated with increased leaf mortality (broken line in Fig. 8).

(ii) Effects of genotypic parameters

The empirical value of *MGR* for IR64 was 1.6, indicating that successively appearing leaves on the main stem are up to 1.6-fold larger (in weight terms) if carbon resources are not limiting their size. *MGR* values below this value led to lower shoot dry weight, plant height and leaf area, but did not affect tiller and leaf number (Fig. 9, top). Under these conditions, leaves remained small, thus limiting both production of assimilates (through poor light interception) and demand for assimilates. Increasing *MGR* above 1.6, however, led to greatly increased plant height, and to a lesser extent SDW and leaf area, while reducing tiller number. Green leaf number was also reduced by high *MGR*, partly because of lower tillering and partly because of increased leaf mortality. Death of old leaves was, in this case, caused by excessive demand for assimilates by large, new leaves during expansion. At extremely high values for *MGR* (e.g. 2.0), competition for assimilates was such that plant height decreased. Increasing *MGR* further killed the plant because of senescence of all leaves (data not presented).

Reducing plastochron below the empirical value for IR64 (60°Cd) increased biomass, leaf area and plant height because of rapid succession of new phytomers (Fig. 9, centre). It also reduced tiller number and led to increased leaf senescence because of severe competition for assimilates among sinks. Conversely, increasing the plastochron (thus, slowing down organogenesis) reduced all aspects of plant growth.

The critical value of *Ic* for tillering (*Ict*) had no effect on growth parameters when it was reduced below the empirical value for IR64 (*Ict* = 1.0) (Fig. 9, bottom). At this level of *Ic*, assimilate supply and demand are equal; permitting the plant to tiller in such situations would mostly lead to deficit situations and would thus cause further decreases of *Ic*. Consequently, *Ict* values below 1 were ineffective. Values higher than 1, however, strongly reduced tiller number, and consequently, leaf number. Leaf area, however, increased slightly as tillering was moderately inhibited (*Ict* between 1.1 and 1.3) because in this interval, reduced leaf mortality set off the effect of reduction in tillering. Larger values of *Ict* decreased leaf area. Lastly, shoot biomass was generally, although moderately, increased by higher values for *Ict*. Note that leaf area and shoot dry weight behaved almost identically when *MGR* and plastochron were varied (Fig. 9, top and centre), but behaved differently under variable *Ict* (Fig. 9, bottom). This was due to accumulation of assimilate reserves in the shoot (up to 33% of dry weight) when tillering was inhibited by high *Ict*, thus decreasing specific leaf area (or increase leaf thickness for a same structural area).

Overall, the strong effect of organogenetic parameters on shoot dry weight simulated by this model is surprising because RUE was constant and partitioning of assimilates

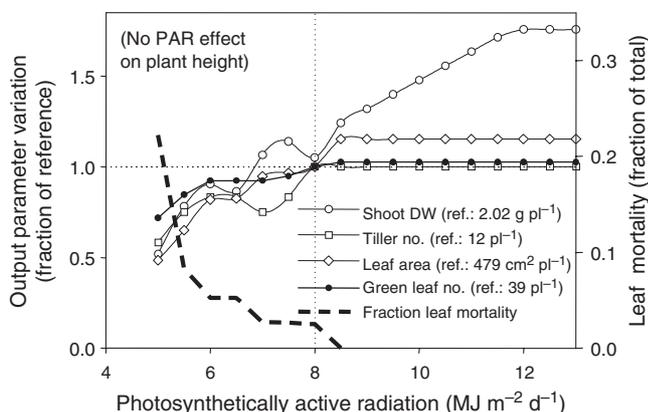


Fig. 8. Sensitivity of various model outputs to various, constant levels of PAR, based on model calibration for IR64 rice and 36-d simulation runs. All model outputs except leaf mortality (left axis) were normalised as fraction of the model output at 8 MJ m⁻² d⁻¹ (experimental conditions in phytotron), with the respective reference values presented in the legend. Leaf mortality is presented as fraction of total leaf number produced (right axis).

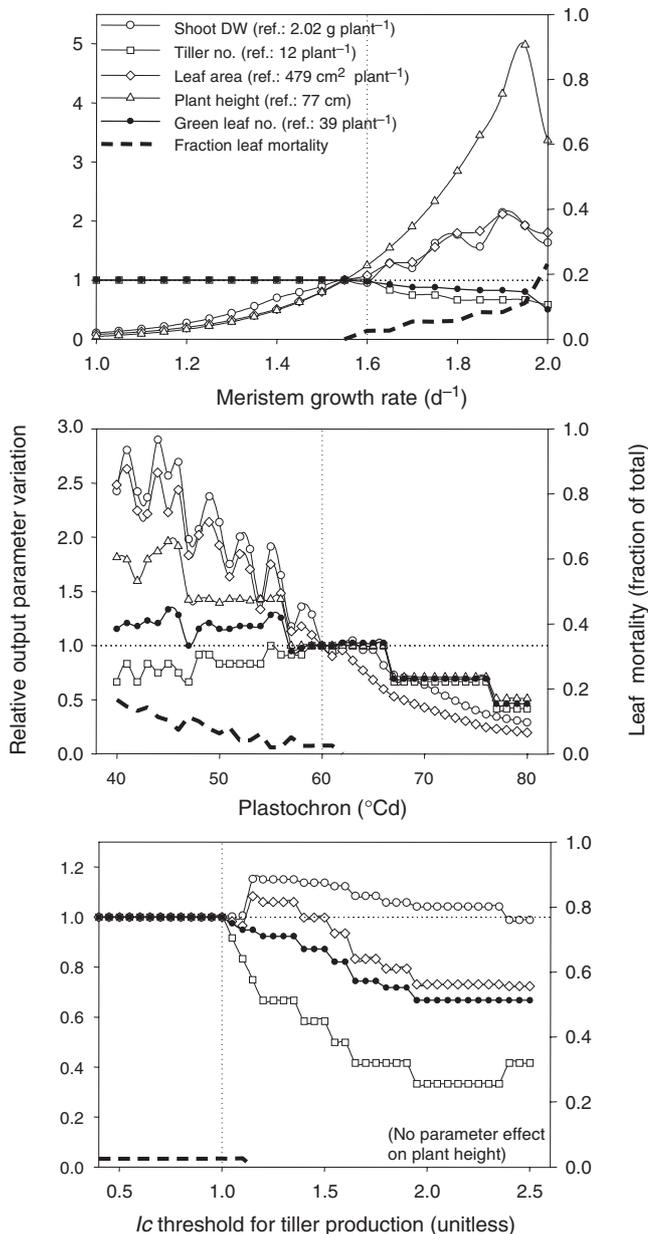


Fig. 9. Sensitivity of various model outputs to variation in values of morphogenetic crop parameters [(top) meristem growth rate; (centre) plastochron; (bottom) I_c threshold for tiller production], based on model calibration for IR64 rice and 36-d simulation runs. As in Fig. 8, crop parameter variation, all model outputs except leaf mortality (left axis) were normalised as fraction of the model output with original IR64 settings at $8 \text{ MJ m}^{-2} \text{ d}^{-1}$ (phytotron conditions), with the respective reference values presented in the legend. Leaf mortality is presented as fraction of total leaf number produced (right axis).

among organs varied little. This apparent paradox was due to the model assumption that assimilates are not necessarily immediately used for growth, but pass through storage pools before the tissues use them for growth. The resulting delays in leaf area production under low-demand conditions, although

small, have a strong effect on growth during its exponential phase. The most significant result of this modelling exercise is thus that plant growth is probably not only driven by assimilate supply, but also by demand for assimilates. We will focus the discussion section of this paper on this hypothesis.

Discussion

The model described here makes use of two well-established concepts, that of growth driven by carbon assimilation (which is at the basis of all agronomic crop models) and that of structural growth, resulting in a tree-type topology (realised in numerous other plant-architectural models). Since emphasis here was on combining the two in order to model interactions between growth and structural development, both complementary concepts were implemented in the simplest possible way. For example, light interception and photosynthesis were calculated at the canopy scale, thereby assuming that Lambert–Beer’s law of light extinction and the concept of RUE (proportionality between light interception and carbon assimilation) are sufficiently accurate to feed into a physiological model of sink–source relationships. In fact, we borrowed these modules from an existing, agronomic crop model (SARRA-H, Dingkuhn *et al.* 2003; Sultan *et al.* 2005), with the result that feedbacks of morphological change on assimilation are essentially mediated by LAI. We acknowledge that the *EcoMeristem* model’s potential would be more fully exploited if plant photosynthesis were also sensitive to changes and distribution within the canopy of SLA, leaf age, nitrogen content (Dingkuhn *et al.* 1992) and leaf orientation and distribution in space (Dauzat 1994; Dauzat *et al.* 2001). Most of these feedbacks are under study for the next version of *EcoMeristem*, but the simplifications and compromises made in the present model bear little on the main result, which is that crop growth depends as much on assimilate supply as it does on internal demand for assimilates.

We hypothesise that the concept guiding most agronomic crop models, namely, that plants generally convert into biomass all resources available to them in the most efficient way, is in many cases wrong. There are numerous examples to the contrary, such as the case of hybrid vigour, which is mostly not related to higher leaf photosynthetic rates, nor to different crop architecture when compared to similar, high-yielding inbred lines (Laza *et al.* 2001). Another, more extreme example is the physiology of temperate, perennial plants, which constitutionally have long lag phases between assimilate production and their re-investment in growth processes, involving large reserve compartments to buffer the asynchrony between supply and demand (Lechaudel *et al.* 2005). Evidently, annual crops bred for rapid growth and maximal production, such as modern cereals, probably have minimal lag periods between assimilate acquisition and their re-investment in resource uptake (including carbon, but also

water and mineral nutrients). This modelling exercise shows, however, that even small imbalances between aggregate source and sink capacity, buffered by transitory storage in vegetative tissues, can have a strong effect on overall biomass growth rate. This effect can be expected to be particularly strong during exponential growth, where any delay or inefficiency in re-investment of internal resources leads to reduced relative growth rate (RGR, Osaki and Shinano 2001). Demand (sink) limitation of vegetative growth would not necessarily disappear at higher plant populations (and therefore, greater competition) because the plant continuously adjusts demand to supply, for example through tillering rate and leaf size. There may thus be intrinsically more demand- or more supply-limited plant types.

Further research is needed to confirm our hypotheses. The present study demonstrated the presence of significant amounts of carbon reserves (sucrose and starch) in vegetative tissues of rice even during exponential growth, but the information is insufficient to quantitatively relate simulated to observed reserve dynamics. Observations were only made at the beginning of the photoperiod, when the transitory reserve pool can be expected to be smallest (Samonte *et al.* 2001), and we neither know the mean pool size for the 24-h cycle, nor can we be sure that the few sugar compounds analysed here capture the totality of reversible storage. Furthermore, a solid proof of concept can only be obtained from observations on constitutionally more or less vigorous genotypes, because according to *EcoMeristem*, superior vigour (on the basis of similar architecture and RUE) should be associated with lower transitory reserve levels. Lastly, it must be remembered that carbon assimilation is not always the main process limiting growth. Consequently, mineral deficiencies and biophysical stresses should, in many situations, increase carbon reserve pools in the plant or, conversely, genotypes adapted to specific mineral deficiencies or stresses may use carbon less efficiently because they don't need to. These hypotheses are under investigation and will be the subject of subsequent papers.

We justified the development of *EcoMeristem* with the need for models that are capable of linking crop phenotypic plasticity in the field to genomic or genetic information. No evidence can be provided at this stage that this model is better suited to this purpose than classical, agronomic models of cereals, which are generally resource driven during vegetative growth. *EcoMeristem* operates with a new type of crop parameters governing morphogenetic reaction norms to internal resources (in addition to classical crop parameters such as *Tb*, *RUE*, *Kdf* or seed size). These new parameters characterise meristem behaviour and are sensitive to sugar signalling, and are thus in line with recent findings on the genetic control (e.g. expression of cell wall invertase genes; Ji *et al.* 2005) and physiological regulation (sugar and hormonal signalling; Black *et al.* 1995) of sinks. Further

research is in progress to explore the relationships of genotypic model parameters with the expression of candidate genes and the activity of key enzymes encoded by them, such as cell wall invertases.

Conclusion

This paper presented a new model, *EcoMeristem*, which simulates interactions between development and growth processes in vegetative rice plants. The underlying hypothesis was that supply of assimilates feeds back on demand for assimilates resulting from the production of new organs and conversely, organ production feeds back on supply (assimilation). Imbalances between instantaneous supply and demand levels are buffered by reserve storage and mobilisation, as well as facultative organ initiation or senescence. Sensitivity analysis of the model suggests that biomass growth may be as much driven by internal demand as by supply. This finding requires further validation. Once the capability of the model to accurately simulate the plant type and phenotypic plasticity of contrasting genotypes has been demonstrated, it will be used to associate model parameters with genetic information.

Acknowledgments

The authors thank the Global Challenge Program (GCP) 'Generation' and Cirad's ORYZON project for funding this research.

References

- Andrade FH, Vega C, Uhart S, Cirilo A, Cantarero M, Valentinuz O (1999) Kernel number determination in maize. *Crop Science* **39**, 453–459.
- Asai K, Satoh N, Sasaki H, Satoh H, Nagato Y (2002) A rice heterochronic mutant, *mori1*, is defective in the juvenile–adult phase change. *Development* **129**, 265–273.
- Asch F, Sow A, Dingkuhn M (1999) Reserve mobilization, dry matter partitioning and specific leaf area in seedlings of African rice cultivars differing in early vigor. *Field Crops Research* **62**, 191–202. doi: 10.1016/S0378-4290(99)00020-9
- Black CC, Loboda T, Chen JQ, Sung SJS (1995) Can sucrose cleavage enzymes serve as markers for sink strength and is sucrose a signal molecule during plant sink development. In 'Proceedings of the first international symposium on sucrose metabolism' (Eds HG Pontis, GL Salerno, EJ Echeverria) pp. 49–64. (American Society of Plant Physiologists: Rockland, MD)
- Boehringer SA (1984) 'Methods of enzymatic food analysis using single reagents'. (Boehringer Mannheim GmbH: Mannheim, Germany)
- Brisson N, Mary B, Ripoche D, Jeuffroy MH, Ruget F, Gate P, Devienne-Barret F (1998) STICS: a generic model for the simulation of crops and their water and nitrogen balance. I. Theory and parameterization applied to wheat and corn. *Agronomie* **18**, 311–346.
- Cookson SJ, van Lijsebettens M, Granier C (2005) Correlation between leaf growth variables suggest intrinsic and early controls of leaf size in *Arabidopsis thaliana*. *Plant, Cell & Environment* **28**, 1355–1366. doi: 10.1111/j.1365-3040.2005.01368.x
- Dauzat J (1994) Radiative transfer simulation on computer models *Elaeis guineensis*. *Oléagineux* **49**, 81–90.

- Dauzat J, Luquet D, Bégué A (2001) Energy balance modelling and thermography simulation on 3D plant models. Application on cotton trees. In 'Proceedings of the third international workshop on functional-structural tree and stand models. Val-Morin, Quebec'. p. 14.
- Dingkuhn M, Miezan KM (1995) Climatic determinants of irrigated rice performance in the Sahel. II. Validation of photothermal constants and characterization of genotypes. *Agricultural Systems* **48**, 411–434. doi: 10.1016/0308-521X(94)00028-J
- Dingkuhn M, Kropff M (1996) Rice. In 'Photoassimilate distribution in plants and crops: source-sink relationships'. (Eds E Zamski, AA Schaffer) pp. 519–547. (Marcel Dekker, Inc.: New York)
- Dingkuhn M, De Datta SK, Pamplona R, Javellana C, Schnier HF (1992) Effect of late-season N fertilization on photosynthesis and yield of transplanted and direct-seeded tropical flooded rice. II. A canopy stratification study. *Field Crops Research* **28**, 235–249.
- Dingkuhn M, Johnson DE, Sow A, Audebert AY (1999) Relationship between upland rice canopy characteristics and weed competitiveness. *Field Crops Research* **61**, 79–95. doi: 10.1016/S0378-4290(98)00152-X
- Dingkuhn M, Baron C, Bonnal V, Maraun F, Sarr B, Sultan B, Clopes A, Forest F (2003) Decision support tools for rainfed crops in the Sahel at the plot and regional scales. In 'Decision support tools for smallholder agriculture in sub-Saharan Africa—a practical guide'. (Eds TE Struiff Bontekes, MCS Wopereis) pp. 127–139. (International Fertilizer Development Center and ACP-EU Technical Centre for Agricultural and Rural Cooperation: Wageningen, The Netherlands)
- Dingkuhn M, Luquet D, Quiot B, Reffye PD (2005) Environmental and genetic control of morphogenesis in crops: towards models simulating phenotypic plasticity. *Australian Journal of Agricultural Research* **56**, 1289–1302. doi: 10.1071/AR05063
- Dubcovsky J (2004) Marker-assisted selection in public breeding programs: the wheat experience. *Crop Science* **44**, 1895–1898.
- Fournier C, Durand JL, Ljutovac S, Schäufele R, Gastal F, Andrieu B (2005) A functional-structural model of elongation of the grass leaf and its relationships with the phyllochron. *New Phytologist* **166**, 881–894. doi: 10.1111/j.1469-8137.2005.01371.x
- Frey JE, Frey B, Sauer C, Kellerhals M (2004) Efficient low-cost DNA extraction and multiplex fluorescent PCR method for marker-assisted selection in breeding. *Plant Breeding* **123**, 554–557. doi: 10.1111/j.1439-0523.2004.01033.x
- Gambin BL, Borrás L, Otegui ME (2004) Maize potential kernel weight: relation with plant growth rate around silking. In 'Proceedings of the 4th international crop science conference, Brisbane, Australia'. Available at www.crops-science.org.au [Verified 19 January 2006]
- GCP (2005) Generation challenge programme — cultivating plant diversity for the resource poor. Available at www.generationcp.org [Verified 19 January 2006]
- Hammer GL, Kropff MJ, Sinclair TR, Porter JR (2002) Future contributions of crop modelling — from heuristics and supporting decision making to understanding genetic regulation and aiding crop improvement. *European Journal of Agronomy* **18**, 15–31. doi: 10.1016/S1161-0301(02)00093-X
- Hanada K (1993) Chapter 4. Tillers. In 'Science of the rice plant'. pp. 222–258. (Food and Agriculture Policy Research Center: Tokyo)
- Hasegawa T, Koroda Y, Seligman NG, Horie T (1994) Response of spikelet number to plant nitrogen concentration and dry weight in paddy rice. *Agronomy Journal* **86**, 673–676.
- Heyer AG, Raap M, Schroeder B, Marty B, Willmitzer L (2004) Cell wall invertase expression at the apical meristem alters floral, architectural, and reproductive traits in *Arabidopsis thaliana*. *The Plant Journal* **39**, 161–169. doi: 10.1111/j.1365-313X.2004.02124.x
- IRRI (2005) Rice knowledge bank. IRRI, International Rice Research Institute, Los Baños, Philippines. Available at www.knowledgebank.irri.org [Verified 19 January 2006]
- Itoh JI, Hasegawa A, Kitano H, Nagato Y (1998) A recessive heterochronic mutation, plastochron 1, shortens the plastochron and elongates the vegetative phase in rice. *The Plant Cell* **10**, 1511–1521. doi: 10.1105/tpc.10.9.1511
- Itoh JI, Nonomura KI, Ikeda K, Yamaki S, Inukai Y, Yamagishi H, Kitano H, Nagato Y (2005) Rice plant development: from zygote to spikelet. *Plant & Cell Physiology* **46**, 23–47. doi: 10.1093/pcp/pci501
- Ji X, Van den Ende W, Van Laere A, Cheng S, Bennett J (2005) Structure, evolution, and expression of the two invertase gene families of rice. *Journal of Molecular Evolution* **60**, 615–634. doi: 10.1007/s00239-004-0242-1
- Jitla DS, Rogers GS, Seneweera SP, Basra AS, Oldfield RJ, Conroy JP (1997) Accelerated early growth of rice at elevated CO₂: is it related to developmental changes in shoot apex? *Plant Physiology* **115**, 15–22.
- Jones JW, Hoogenboom G, Porter CH, Boote KJ, Batchelor WD *et al.* (2003) The DSSAT cropping system model. *European Journal of Agronomy* **18**, 235–265. doi: 10.1016/S1161-0301(02)00107-7
- Kiniry JR, McCauley G, Xie Y, Arnold JG (2001) Rice parameters describing crop performance of four U.S. cultivars. *Agronomy Journal* **93**, 1354–1361.
- Kobayashi K, Horie Y, Imaki T (2002) Relationship between apical dome diameter at panicle initiation and the size of panicle components in rice grown under different nitrogen conditions during the vegetative stage. *Plant Production Science* **5**, 3–7.
- Kropff M, van Laar HH (1993) 'Modelling crop-weed interactions.' (CAB International: Cambridge)
- Kropff MJ, Cassman KG, Peng S, Matthews RB, Setter TL (1994) Quantitative understanding of yield potential. In 'Breaking the yield barrier'. (Ed. KG Cassman) pp. 21–38. (International Rice Research Institute: Los Baños, Philippines)
- Laza MRC, Peng S, Sanico AL, Visperas RM, Akita S (2001) Higher leaf area growth rate contributes to greater vegetative growth of F1 rice hybrids in the tropics. *Plant Production Science* **4**, 184–188.
- Lechaudel M, Genard M, Lescouret F, Urban L, Jannoyer M (2005) Modeling effects of weather and source-sink relationships on mango fruit growth. *Tree Physiology* **5**, 583–597.
- Luquet D, Zhang B, Dingkuhn M, Dext A, Clément-Vidal A (2005) Phenotypic plasticity of rice seedlings: case of phosphorus deficiency. *Plant Production Science* **8**, 145–151. doi: 10.1626/pp.s.8.145
- Miyoshi K, Ahn BO, Kawakatsu T, Ito Y, Itoh JI, Nagato Y, Kurata N (2004) *PLASTOCHRON1*, a timekeeper of leaf initiation in rice, encodes cytochrome P450. *Proceedings of the National Academy of Sciences USA* **101**, 875–880. doi: 10.1073/pnas.2636936100
- Monteith JL (1994) Validity of the correlation between intercepted radiation and biomass. *Agricultural and Forest Meteorology* **68**, 213–220. doi: 10.1016/0168-1923(94)90037-X
- Moreau L, Charcosset A, Gallais A (2004) Experimental evaluation of several cycles of marker-assisted selection in maize. *Euphytica* **137**, 111–118. doi: 10.1023/B:EUPH.0000040508.01402.21
- Munns R, Weir R (1981) Contribution of sugars to osmotic adjustment in elongating and expanded zones of wheat leaves during moderate water deficits at two light levels. *Australian Journal of Plant Physiology* **8**, 93–105.
- Murchie EH, Hubbart S, Peng S, Horton P (2005) Acclimation of photosynthesis to high irradiance in rice: gene expression and interaction with leaf development. *Journal of Experimental Botany* **56**, 449–460. doi: 10.1093/jxb/eri100

- Nelder J, Mead R (1965) A simplex method for function minimization. *Computer Journal* **7**, 308–313.
- Osaki M, Shinano T (2001) Plant growth based on interrelation between carbon and nitrogen translocation from leaves. *Photosynthetica* **39**, 197–203. doi: 10.1023/A:1013770807583
- Penning de Vries FWT, Jansen HFM, Bakema A (1989) Simulation of ecophysiological processes of growth in several annual crops. (Pudoc: Wageningen, The Netherlands)
- Reynolds M, Condon AG, Rebetzke GJ, Richards RA (2004) Evidence for excess photosynthetic capacity and sink-limitation to yield and biomass in elite spring wheat. In 'Proceedings of the 4th international crop science congress, Brisbane, Australia. Available at [www.cropscience.org.au](http://www.cropsscience.org.au) [Verified 19 January 2006]
- Reynolds MP, Pellegrineschi A, Skovmand B (2005) Sink-limitation to yield and biomass: a summary of some investigations in spring wheat. *Annals of Applied Biology* **146**, 39–49.
- Samonte SOPB, Wilson LT, McClung AM, Tarpley L (2001) Seasonal dynamics of nonstructural carbohydrate partitioning in 15 diverse rice genotypes. *Crop Science* **41**, 902–909.
- Schnier HF, Dingkuhn M, De Datta SK, Mengel K, Faronilo JE (1990) Response of direct seeded and transplanted flooded rice to nitrogen fertilization: I. Plant nitrogen uptake, canopy photosynthesis, growth and yield. *Crop Science* **30**, 1276–1284.
- Sherson SM, Alford HL, Forbes SM, Wallace G, Smith SM (2003) Roles of cell-wall invertases and monosaccharide transporters in the growth and development of *Arabidopsis*. *Journal of Experimental Botany* **54**, 525–531. doi: 10.1093/jxb/erg055
- Skinner RH, Nelson CJ (1995) Elongation of the grass leaf and its relationship to the phyllochron. *Crop Science* **35**, 4–10.
- Sultan B, Baron C, Dingkuhn M, Sarr B, Janicot S (2005) Agricultural impacts of large-scale variability of the West African monsoon. *Agricultural and Forest Meteorology* **128**, 93–110. doi: 10.1016/j.agrformet.2004.08.005
- Sylvester AW, Parker-Clark V, Murray GA (2001) Leaf shape and anatomy as indicators of phase change in the grasses: comparison of maize, rice, and bluegrass. *American Journal of Botany* **88**, 2157–2167.
- Tardieu F, Reymond M, Hamard P, Granier C, Muller B (2000) Spatial distributions of expansion rate, cell division rate and cell size in maize leaves: a synthesis of the effects of soil water status, evaporative demand and temperature. *Journal of Experimental Botany* **51**, 1505–1514. doi: 10.1093/jexbot/51.350.1505
- Tivet F, da Silveira Pinheiro B, de Raïssac M, Dingkuhn M (2001) Leaf blade dimensions of rice (*Oryza sativa* L. and *Oryza glaberrima* Steud.). Relationships between tillers and main stem. *Annals of Botany* **88**, 507–511.
- Verhoerf W (1985) Light scattering by leaf layers with application to canopy reflectance modelling: the SAIL model. *Remote Sensing of Environment* **17**, 1666–1678.
- Verma AK, Pandey N, Tripathi RS (2004) Leaf growth, chlorophyll, nitrogen content and grain yield of hybrid rice as influenced by planting times and N levels. *Annals of Agricultural Research* **25**, 456–458.
- Walter A, Schurr U (2005) Dynamics of leaf and root growth: endogenous control versus environmental impact. *Annals of Botany* **95**, 891–900. doi: 10.1093/aob/mci103
- Wang E, Robertson MJ, Hammer GL, Carberry PS, Holzworth D, Meinke H, Chapman SC, Hargreaves JNG, Huth NI, McLean G (2002) Development of a generic crop model template in the cropping system model APSIM. *European Journal of Agronomy* **18**, 121–140. doi: 10.1016/S1161-0301(02)00100-4
- Williams RF (1975) 'The shoot apex and leaf growth. A study in quantitative biology.' (Cambridge University Press: London)
- Wright SD, McConnaughay KDM (2002) Interpreting phenotypic plasticity: the importance of ontogeny. *Plant Species Biology* **17**, 119–131. doi: 10.1046/j.1442-1984.2002.00082.x
- Yan HP, Kang MZ, Reffye PD, Dingkuhn M (2004) A dynamic, architectural plant model simulating resource-dependent growth. *Annals of Botany* **93**, 591–602. doi: 10.1093/aob/mch078
- Yoshida H, Horie T, Shiraiwa T (2006) A model explaining genotypic and environmental variation of spikelet number per unit area measured by cross-locational experiments in Asia. *Field Crops Research* (In press).

Manuscript received 31 October 2005, accepted 18 January 2006

***EcoMeristem*, a model of morphogenesis and competition among sinks in rice. 2. Simulating genotype responses to phosphorus deficiency**

Michael Dingkuhn^{A,B}, Delphine Luquet^A, HaeKoo Kim^A, Ludovic Tambour^A
and Anne Clement-Vidal^A

^ACIRAD, Amis Department, TA40/01 Av. Agropolis, 34398 Montpellier Cedex 5, France.

^BCorresponding author. Email: dingkuhn@cirad.fr

Abstract. Phenotypic plasticity enables plants to adjust their morphology and phenology to variable environments. Although potentially important for crop breeding and management, the physiology and genetics of plasticity traits are poorly understood, and few models exist for their study. In the previous paper of this series, the structural–functional model *EcoMeristem* was described and field validated for vegetative-stage rice. This study applies the model to an experimental study on phosphorus deficiency effects on two morphologically contrasting rice cultivars, IR64 and Azucena, grown in controlled environments under hydroponics culture. Phosphorus deficiency caused severe biomass growth reductions in the shoot but not in the root, thus increasing the root / shoot weight ratio. It also inhibited tiller formation and leaf elongation, prolonged the phyllochron, and increased carbohydrate reserve pools in the plant. Analysis aided by the model identified inhibition of leaf extension and tillering as primary effects of the stress. Physiological feedback probably led to longer phyllochron, greater reserve accumulation and root growth stimulation. The main effect of P deficiency appeared to be a reduction in demand for assimilates in the shoot while photosynthetic radiation use efficiency remained nearly constant, resulting in spill-over of excess assimilates into reserve compartments and root growth. The results are discussed in the light of future applications of *EcoMeristem* for phenotyping and genetic analyses of phenotypic plasticity.

Keywords: carbohydrates, leaf extension rate, *Oryza sativa* L., phyllochron, root / shoot ratio, tillering.

Introduction

Genotype \times environment interaction ($G \times E$) is a term commonly used by breeders and geneticists to describe phenotypic variation that can be neither explained by genotype nor by environment variation alone (Brancourt-Hulmel and Lecomte 2003). It thus quantifies the bulk, unexplained, observed variation but provides no information on the nature and possible adaptive value of this variation. Furthermore, statistical $G \times E$ is based on large numbers of genotype and environment combinations, and therefore does not permit extrapolation of individual genotypes' behaviour to specific environments. Statistical (black box) $G \times E$ terms are useful in conventional breeding but are less helpful in the development of gene-specific markers derived from candidate genes having a known function. Researchers investigating phenotypic expression of genes and their polymorphisms prefer describing $G \times E$

as phenotypic plasticity (Dingkuhn 1996; Wright and McConnaughay 2002; Luquet *et al.* 2005a), which is the result of variable expression of the genes concerned, their interaction with the effects of other genes, and physiological interactions among gene products within the plant system.

From a physiological angle, phenotypic plasticity is the environment-induced diversity of phenotypes a given genotype can generate, brought about by the responsiveness of the plant's metabolic, growth and developmental processes to external and internal signals. This responsiveness may or may not involve changes in the expression patterns of genes, depending on whether the response is actively induced or inherent (constitutive) to the physiological apparatus. Phenotypic plasticity rarely affects the body plan (or basic structure) of the phenotype, but can strongly affect morphology (Dingkuhn 1996; Bos and Neuteboom 1998),

Abbreviations used: CIN, cell wall invertase; DAT, days after transplanting; $G \times E$, genotype \times environment interaction; *Ic*, internal competition index; LER, leaf extension rate; MAP, model assisted phenotyping; PAI, plant area index; RSR, root / shoot DW ratio; RUE, radiation-use efficiency; Ta, daily mean air temperature; Tb, base temperature; TT, thermal time.

histology (Jitla *et al.* 1997) and phenology (Clerget *et al.* 2004) of a plant.

In this study, we use P deficiency as environmental factor to modify the phenotype of two rice cultivars that have different morphology, namely in terms of tiller and leaf number, plant height and leaf size. Phosphorus deficiency is a widespread constraint to cereal production, particularly in weathered, acid, tropical soils (Reich 2001; Ming *et al.* 2002; Wissuwa 2003). Wissuwa and Ae (2001) showed that tolerance to P deficiency mainly depends on the maintenance of P uptake rate. In tolerant genotypes, this is achieved with physiological adaptations (increasing P uptake per root area) and morphological adaptations (increasing access to soil P and reducing shoot P requirements, Wissuwa 2003). The affected morphogenetic processes include reductions in shoot growth, tiller production and leaf appearance rate, while root growth and root branching are stimulated (Ming *et al.* 2002; Shane *et al.* 2003). Since these responses involve specific modifications of architecture (e.g. branching), phenology (e.g. phyllochron) and assimilate partitioning (root/shoot weight ratio), they represent an interesting ensemble for the study of phenotypic plasticity. Recently, Luquet *et al.* (2005a, b) reported that morphogenetic effects of P deficiency in rice are related to differential sugar allocation among growing organs, and between growing organs and reserve compartments. The present study investigates these relationships using a combined experimental and modelling approach. Some other known effects of P deficiency such as root citrate secretion (Shane *et al.* 2003) and phosphatase secretion (Ming *et al.* 2002), which enhance the mobilisation of soil P resources, were not studied because emphasis here is on morphogenetic processes.

In part 1 of this series (Luquet *et al.* 2006), we introduced and validated the model *EcoMeristem*, which simulates resource-dependent growth and morphogenesis of rice, on the basis of internal competition relationships among growing organs (sinks). According to this model, organogenetic processes, which create and dimension new sinks, are affected by the balance between supply and demand of plant carbon assimilate, a concept inspired by recent findings on sugar signalling (Black *et al.* 1995; Roitsch *et al.* 2000). Assimilate supply thus affects demand, and demand feeds back on fresh assimilate production through organ growth. The model was developed to assist phenotyping of complex traits, such as growth responses to environment, for QTL development and association mapping. Such heuristic approaches (Chapman *et al.* 2002; Hammer *et al.* 2002) have recently been explored with models describing the whole plant derived from agronomic crop models (Yin *et al.* 2004) or detailed models describing a specific set of physiological processes (Reymond *et al.* 2003, 2004). Dingkuhn *et al.* (2005) discussed the potential usefulness of different types of plant models for model-assisted phenotyping (MAP) and concluded that advances can be

expected from new types of whole-plant models simulating phenotypic plasticity, in order to (i) obtain crop parameters that are less prone to $G \times E$ 'noise' but instead, explain some of it, and (ii) measure the impact of model parameter variation on plant responses to variable environments (Chapman *et al.* 2002; Boote *et al.* 2003; Hoogenboom and White 2003). It is also expected that model parameters calibrating biological processes, as opposed to parameters forcing phenotype features directly, are functionally closer to gene action. Model-assisted phenotyping may thus give better access to major QTLs or the functional analysis of candidate-gene polymorphisms than phenotyping based on the measurements conventionally made by breeders (e.g. leaf area index or yield).

This paper aims to analyse the phenotypic plasticity exhibited by rice under P deficiency, and the capability of the *EcoMeristem* model to simulate this plasticity on the basis of genotype specific crop parameters. We thereby do not attempt to study or model P uptake and fluxes within the plant. In fact, by using hydroponics, the experimental design imposes levels of plant P deficiency that are less prone to of plant adaptations that may improve P uptake in the field, such as secretion of substances modifying the rhizosphere. Our focus is thus on physiological and morphological responses of the plant to an imposed deficit condition.

Materials and methods

Experimental design

An experiment was conducted in controlled environments at CIRAD (Montpellier, France) between April and June 2004 with the objectives to characterise morphological and architectural variation (phenotypic plasticity) induced by phosphorus (P) deficiency, relate this plasticity to assimilate partitioning within the plant, and evaluate the capacity of the *EcoMeristem* model to simulate these phenomena. The experiment had two P levels (treatments P+ and P-) and four replicates in a randomised block design. Hydroponics was used to ensure that differential P treatments had full impact on plants while minimising the effect on P uptake of specific physiological adaptations such as citrate and phosphatase secretion by roots, assuming that these were removed daily by irrigation. Two contrasting rice genotypes were used, IR64 (*Oryza sativa* L., indica type) having small leaves and producing a large number of tillers per plant during vegetative development and Azucena (japonica type) having larger leaves and a lower tillering rate.

After seeds were germinated for 4 d at 33°C in an illuminated culture chamber, plants were transplanted in drained, 1-L pots containing quartz sand. Pots were watered daily to field capacity with a culture solution (pH 5.5) containing the following nutrients (concentrations in mM): $\text{KH}_2\text{PO}_4 = 0.21$, $\text{K}_2\text{HPO}_4 = 0.06$, $\text{KNO}_3 = 1.98$, $\text{Ca}(\text{NO}_3)_2 = 2.96$, $\text{MgSO}_4 = 0.61$, $\text{KCl} = 0.1$, $(\text{NH}_4)_2\text{SO}_4 = 0.53$, $\text{MnSO}_4 = 2.9 \times 10^{-3}$, $(\text{NH}_4)_2\text{MoO}_4 = 6 \times 10^{-5}$, $\text{CuSO}_4 = 6.3 \times 10^{-2}$, $\text{ZnSO}_4 = 2.5 \times 10^{-3}$, $\text{H}_3\text{BO}_3 = 7.4 \times 10^{-3}$, $\text{EDTA-Fe} = 0.206$. Air temperature in the culture chamber was 28/23°C (day/night), relative air humidity was 60%/80% (day/night), and PAR was $8.0 \text{ MJ m}^{-2} \text{ d}^{-1}$, supplied with halogen lamps during a 14-h photoperiod.

From 12 d after transplanting (DAT) onwards, half of the plant population was subjected to a P deficiency treatment (P-: $0.009 \text{ mM PO}_4^{3-}$, or 1/30 of control treatment) while the remaining plants continued to receive optimal nutrition (P+: $0.27 \text{ mM PO}_4^{3-}$). According

to Marschner and Vetterlein (1989), P in the soil solution strongly affects plant morphology at and below a concentration of 0.01 mM. Leaf and root P concentrations in the deficiency treatment decreased within 5 d after treatment onset to $\sim 1 \text{ mg g}^{-1}$ (DW based) and fluctuated little thereafter. This concentration corresponds to the critical concentration for severe P deficiency in rice (IRRI 2005).

Destructive measurements on roots and shoots were made on five dates (12, 19, 25, 30 and 36 DAT). Details of the sampling procedure and measurements are described in the companion paper (Luquet *et al.* 2006). Destructive measurements included bulk root and individual leaf sheath and blade dry weight on each culm. The same samples were used for analyses of starch, sucrose, fructose and glucose content by Dionex HPLC (HPLC-PAD detector) after hydrolysis (case of starch) and extraction with 80% ethanol at 80°C. Carbohydrate analyses were only conducted on three of the four available replications.

Non-destructive measurements (twice daily) included observations on leaf appearance on all culms to calculate phyllochron (thermal time separating the appearance of two successive leaves on the main stem), leaf blade and sheath length and width and tiller number. Plant area index (PAI, total leaf blade area in cm^2) was estimated by eqn 1.

$$\text{PAI} = \left(\sum_i L(i) \times W(i) \times 0.725 \right), \quad (1)$$

where $L(i)$ and $W(i)$ are the length and width (cm), respectively, of blade i on a given plant, and 0.725 is an allometric coefficient used to relate L and W to surface area LA (Tivet *et al.* 2001).

Specific leaf area (SLA, $\text{m}^2 \text{g}^{-1}$) of individual blades was computed by eqn 2.

$$\text{SLA} = \text{LAb} / \text{DWb}, \quad (2)$$

where LAb and DWb represent individual leaf blade area (m^2) and dry weight (g), respectively.

Growth and development dynamics were expressed using either thermal time (TT in °Cd, eqn 3) or DAT as reference.

$$\text{TT} = \sum_1^n (\text{Ta} - \text{Tb}), \quad (3)$$

where Ta is the daily mean air temperature (°C) and Tb the base temperature below which plant development stops (assumed to be 13°C; Tivet *et al.* 2001).

EcoMeristem model

A detailed description of model structure, parameterisation procedures and sensitivity to input variables and crop parameters is given in the companion paper (Luquet *et al.* 2006). Only the underlying concepts are repeated here.

EcoMeristem is a whole-plant, deterministic, dynamic, radiation- and temperature-driven crop model (the model also has a soil and plant water balance but these modules were not used in this study). The specificity of the model is its capability to simulate competition for assimilates (aggregate, incremental supply) among growing organs (demand functions). The supply, consisting of daily carbon assimilation minus maintenance respiration, is thereby simulated at the scale of the whole plant (situated within a canopy formed by a homogenous population), whereas demand is simulated at the individual organ level, then aggregated to provide a whole-plant demand term. This procedure allows comparison of plant-level supply and demand for each time step (24 h) and simulation of feedbacks of supply/demand imbalances on organ number (organogenesis), growth rate and final size (morphogenesis). Supply/demand relationships are estimated with a state variable called Ic (Index of internal competition), calculated as aggregate supply divided by aggregate demand for each time step of model execution.

Excess assimilates ($Ic > 1$) are reversibly stored as reserves, or if the reserve compartment is saturated, reduce photosynthesis (product inhibition). Deficient assimilates (low Ic) cause two types of adaptive responses. First ($Ic < 1$), the current assimilate shortfall for growth is buffered by reserve mobilisation, organ senescence (followed by recycling) and ultimately, delays in organogenetic cycles, in this order; and second, organs that are being initiated are down-sized, leading to smaller demand when they turn into sinks. The Ic conditions also branching events, i.e. tiller appearance in the case of rice. This system of feedbacks stabilises plant carbon balance by adjusting plant development to resources.

The model was parameterised using target files containing morphological observations made on the last sampling date (36 DAT, i.e. 38 d after germination), using statistical optimisation procedures in the case of morphogenetic parameters that cannot be measured directly only the plant (Luquet *et al.* 2006). Parameterisation was done individually for each treatment and replication in order to calculate SE of parameter values. The objective was thereby not to use the model to predict plant behaviour (extrapolation), but to study treatment and genotype effects on crop parameters (heuristic approach).

Results

Treatment and genotype effects on model parameters

The complete set of crop parameters was presented for IR64 in part 1 of this study (Luquet *et al.* 2006). Initial parameters describing seed and seedling properties differed for Azucena, which had slightly heavier seed (30 mg; IR64: 28 mg) and first leaf (6 mg; IR64: 4 mg), and slightly lower, initial, specific leaf area (SLA_{ini}) of the first leaf ($0.044 \text{ m}^2 \cdot \text{g}^{-1}$; IR64: $0.047 \text{ m}^2 \cdot \text{g}^{-1}$). Crop parameter values taken from the literature, such as base temperature (Tb), PAR extinction coefficient (K_{df}) and tissue storage capacity of carbohydrate reserves (STORM_{max}), were assumed to have the same values for Azucena and IR64 (details in Luquet *et al.* 2006).

Potential radiation use efficiency (RUE_{pot}), the organogenetic parameters plastochron (PLAS), potential meristem growth rate (MGR), Ic threshold for tiller initiation (Ic_t), as well as the ratio of root v. shoot demand for assimilates (RSR_{dem}) were optimised statistically for each combination of genotype, P treatment and replication (Table 1), in order to evaluate genotype, treatment and combined effects on parameter values.

The parameter value for RUE_{pot} was the same for Azucena and IR64 under P+ supply (2.9 g MJ^{-2}). It was only very slightly reduced under P- ($P < 0.05$ for IR64, n.s. for Azucena). The relatively high observed values of RUE_{pot}, compared to $2.0\text{--}2.3 \text{ g MJ}^{-2}$ commonly found for rice (Kiniry *et al.* 2001) were caused by the inclusion of root growth and maintenance respiration in its calculation, processes that are conventionally not included in RUE.

Azucena had a significantly ($P < 0.05$) higher value for PLAS than IR64, indicating that leaves on the main stem were initiated in slower succession. Phosphorus deficiency further increased PLAS in both cultivars, thus reducing development rate of the plant. The MGR value for Azucena (1.9) was higher than that of IR64 (1.6), indicating that the factor by which

Table 1. Empirical values for key parameters of the *EcoMeristem* model for IR64 and Azucena rice under abundant (P+) and deficient (P-) treatments (means and SE of four replications)

For full set of parameters and details of parameterisation methods refer to the companion paper (Luquet *et al.* 2006). Some of this information, differing slightly because an earlier version of the model was used, has been published previously in a conceptual paper on various modelling approaches (Dingkuhn *et al.* 2005; with the kind permission of *Australian Journal of Agricultural Research*)

| Parameter | IR64 | | | | Azucena | | | |
|--|------|------|------|------|---------|------|------|------|
| | P+ | | P- | | P+ | | P- | |
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Parameters governing carbon acquisition and growth | | | | | | | | |
| <i>RUE</i> _{pot} (potential radiation-use efficiency, g m ⁻² J ⁻¹) | 2.88 | 0.17 | 2.51 | 0.08 | 2.91 | 0.05 | 2.75 | 0.12 |
| Parameters governing organogenesis | | | | | | | | |
| <i>PLAS</i> (plastochron, °Cd) | 47.3 | 1.4 | 58.1 | 2.4 | 55.0 | 0.6 | 76.2 | 2.8 |
| <i>MGR</i> (potential meristem growth rate, PLAS ⁻¹) | 1.60 | 0.08 | 1.61 | 0.08 | 1.92 | 0.08 | 2.00 | 0.10 |
| <i>Ict</i> (<i>Ic</i> threshold for tillering, unitless) | 1.00 | 0.09 | 1.88 | 0.17 | 1.76 | 0.05 | 2.08 | 0.29 |
| Allometric parameters | | | | | | | | |
| <i>RSRdem</i> (Root / shoot assimilate demand ratio) | 0.31 | 0.01 | 0.42 | 0.01 | 0.29 | 0.01 | 0.47 | 0.02 |

successively produced leaves increased in DW was higher in Azucena. Lastly, Azucena had a much higher tillering threshold (*Ict*) than IR64, indicating that Azucena would only produce a new tiller if carbohydrate resources were abundant. Phosphorus deficiency further increased *Ict* for both cultivars, thus inhibiting tiller production.

Both cultivars had the same value for *RSRdem* under abundant P supply, and thus did not differ in assimilate partitioning to roots. Phosphorus deficiency, however, significantly ($P < 0.05$) increased *RSRdem* in both cultivars, and particularly in Azucena.

In summary, according to the empirical model parameters, the two cultivars had the same RUE (a measure of photosynthetic capacity) and root/shoot partitioning ratios, but Azucena had lower tillering ability, longer intervals between successively appearing leaves and larger leaves. Phosphorus deficiency had little effect on photosynthetic capacity and none on leaf size but reduced leaf and tiller initiation rate, while affecting shoot growth more strongly than root growth (or stimulating root growth in relative terms). Consequently, the organogenetic and partitioning parameters of the model (except *MGR*) were sensitive not only to genotype but also to P deficiency, and can thus not be regarded as invariable, genotypic parameters.

Growth and biomass partitioning

Under abundant P supply, both cultivars produced about the same shoot biomass during the observed period, but IR64 produced three times as many basal tillers as did Azucena (Fig. 1), including primary, secondary and tertiary tillers. Phosphorus deficiency decreased tiller production by ~70% in IR64 (the high-tillering genotype) and by ~40% in Azucena. It also reduced shoot dry matter production by nearly two-thirds in both cultivars. The model, calibrated on the observations made on the last sampling date (36 DAT), reproduced satisfactorily the kinetics of dry weight growth and tillering.

The measured DW ratio between root and shoot systems was ~0.6 in both cultivars at the time when germination was completed (first leaf after the coleoptile fully expanded) and the seedlings were transplanted (data not presented). At 12 d after transplanting, the root/shoot DW ratio was between 0.25 and 0.3 and then changed little under abundant P supply, but increased markedly under P deficiency. The model, due to treatment-specific calibration, reproduced these kinetics accurately (Fig. 2).

Absolute DW of root systems per plant at 30 DAT, when P treatment effects were fully expressed, was slightly smaller under P deficiency than in controls (reductions of -17% in IR64 and -15% in Azucena) (Table 2). The greater DW fraction allocated to roots under P deficiency (~32% compared to 21% in controls) did thus not offset the even stronger overall growth inhibition.

Leaf appearance and extension rates

The observed phyllochron (thermal time elapsing between two successively appearing leaf tips) was constant during the developmental stages observed on control plants, as indicated by a linear relationship between leaf number and thermal time (Fig. 3). Leaf appearance rates can be calculated as the slope of this function, which was 0.0169 leaves °Cd⁻¹ for IR64 and 0.0147 leaves °Cd⁻¹ for Azucena under abundant P supply. Phosphorus deficiency increased phyllochron, and conversely, reduced leaf appearance rates to 0.0125 leaves °Cd⁻¹ (IR64) and 0.0104 leaves °Cd⁻¹ (Azucena). The relative reduction of leaf appearance rate induced by P deficiency was 26% for IR64 and 29% for Azucena, indicating that varietal and stress effects were additive, and not interactive.

Despite different leaf appearance rates (and therefore, different elongation durations because in rice, the rapid elongation of leaf *n* stops when leaf *n*+1 appears; Nemoto *et al.* 1995; Luquet *et al.* 2006), final leaf blade size was not affected by P deficiency. Consequently, leaf

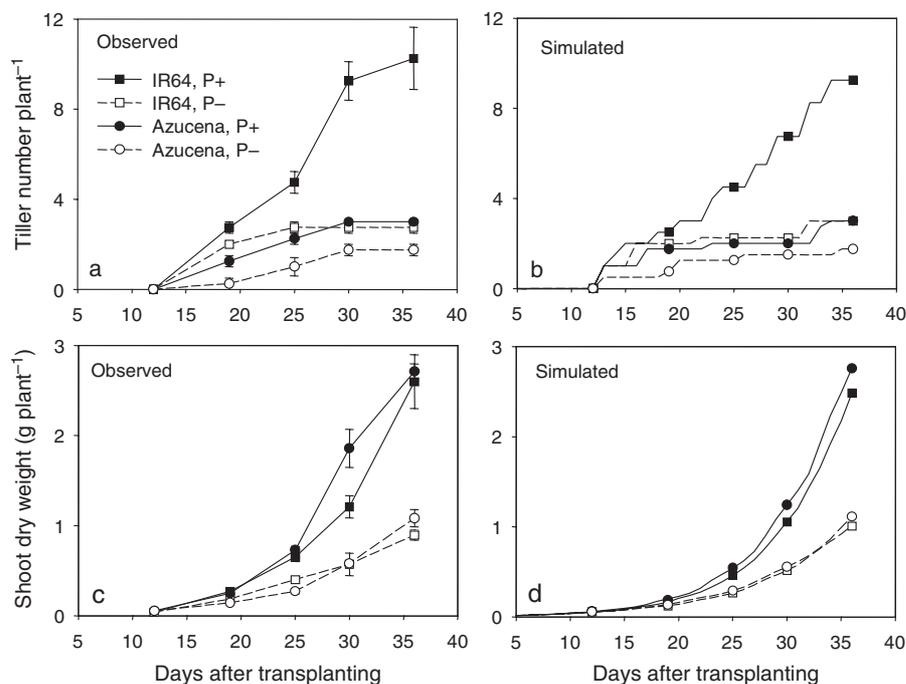


Fig. 1. Observed and simulated time courses of tiller number (*a, b*) and shoot DW per plant (*c, d*) for Azucena and IR64 rice observed under abundant P supply (P+) and deficient supply (P-). Vertical error bars indicate standard error of four replications.

extension rates (LER) observed between leaf tip appearance and ligulation were strongly affected by genotype and P treatment (Fig. 4).

Calculation of LER from plant observations was done in two different ways. The mean LER was obtained by dividing the final leaf blade length by the time elapsing between tip and ligule appearance (corresponding to a phyllochron), and the maximal LER was calculated from daily observations on the length of the appeared portion of the leaf. In both cases, it was assumed that the previous leaf's sheath, from which the tip of the new leaf appeared, had already attained its final length, and that no internode elongation took place, which would have pushed the apex upwards and caused an erroneous LER. Measurements of sheath length and apex position confirmed these assumptions (data not presented).

Mean LER increased approximately linearly with leaf position, whereas maximum LER increased linearly for the first four leaves and then levelled off, or even decreased. These findings are not necessarily conflicting because they integrate different periods of observation. As an observation common to both methods applied, Azucena generally had higher LER than had IR64, and P deficiency reduced LER for both cultivars. The model accurately reproduced the observed differences in mean LER, but it simulated a non-linear (progressive) increase in LER with leaf position, as opposed to the nearly linear pattern observed. It will therefore require some modifications in the way the MGR parameter is implemented (which governs size relationships among leaf

positions), particularly for simulations of longer periods of growth that involve larger number of leaves.

Carbohydrate dynamics

Observed patterns of carbohydrate distribution and dynamics were quite similar in both cultivars and we present details only for Azucena (Fig. 5). Sucrose concentration in leaf blades was constant across sampling dates at approximately 70 mg g^{-1} and was not affected by P treatment. Glucose concentration in leaf blades, which was much lower than that of sucrose, increased steadily during plant development. This increase was strongly inhibited by P deficiency, a phenomenon that was probably related to the reduced growth rate of leaves observed at the same time. Fructose concentration was generally proportional to glucose concentration, the ratio being $\sim 1:1$ in shoot organs (leaf blades and sheaths) and $2:1$ in roots (Fig. 5*i*, inset graph).

In the leaf sheaths, sucrose concentrations were less stable than in blades (Fig. 5). They showed a decreasing trend in control plants, but not in P-deficient plants, resulting in higher sucrose concentrations relative to controls. A similar, but less significant increase in starch concentration was also observed in sheaths under P deficiency.

Glucose concentrations in roots varied little during the experiment and were not significantly affected by P treatment. Sucrose concentrations in roots, however, were significantly increased by P deficiency. This applied also to starch, although concentrations of this compound were extremely

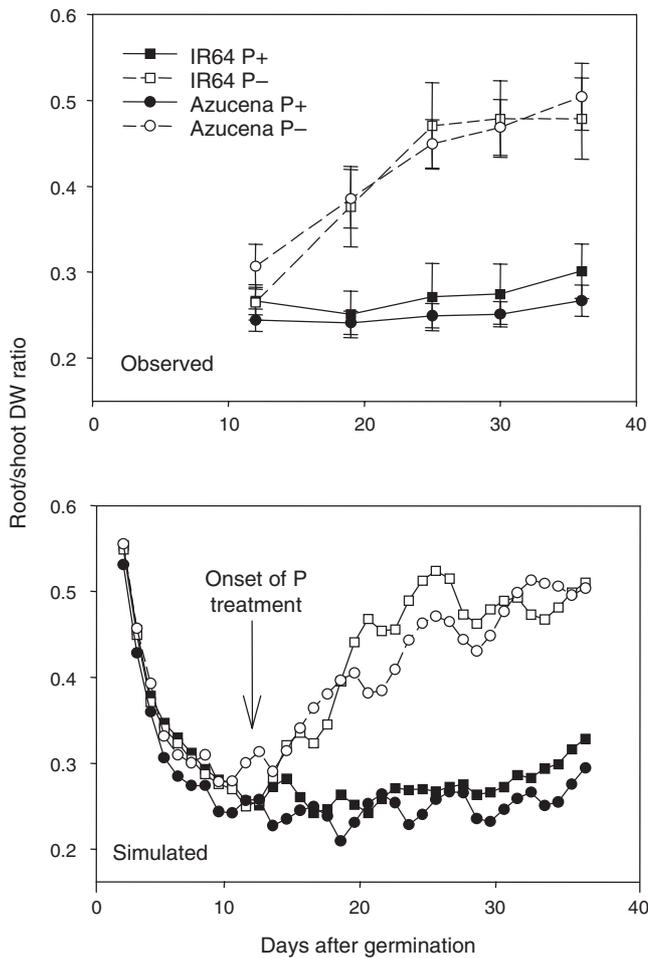


Fig. 2. Observed and simulated root/shoot DW ratio for IR64 and Azucena rice grown under abundant P supply (P+) and deficient supply (P-). Vertical error bars indicate standard error of four replications.

small in roots. Absolute, total sugar content of root systems at 30 d after transplanting, when treatment effects were fully expressed, did not differ significantly between the treatments, although the P deficiency treatment nearly doubled the fraction of plant sugar content found in roots (Table 2).

In summary, leaf blades whose growth was inhibited by P deficiency had markedly decreased concentrations of hexoses. Such a decrease was not observed in roots, whose growth was stimulated by P deficiency in relative terms (compared with shoot growth). However, P deficiency caused a marked increase in sucrose and starch concentration in sheaths and roots.

The model does not permit simulation of specific carbohydrate compounds, but it distinguishes between assimilate incorporated into structural biomass (supply) and reserves (here defined as carbohydrates that are not immediately invested in structural growth). According to the model, assimilate resources (and thus, I_c) are high initially due to seed reserves (Fig. 6). I_c then attains a steady-state equilibrium, fluctuating between 0.8 (deficit) and 2 (abundance), the oscillations being caused by organogenetic (and thus carbon demand) cycles that produce cohorts of leaves and tillers. This steady-state level is slightly higher for P-deficient plants than for control plants because of general carbon demand decrease due to lower leaf initiation rates and a higher I_{ct} threshold for tillering. As a result, higher levels of transitory assimilate reserves in the shoot are simulated for P deficient plants. The simulated patterns of reserves resembled those of observed starch and sucrose concentration in the sheath, although the model seemed to over-estimate the reserve compartment. The measurements of starch and sucrose, measured in the morning, however, represent only trend information because diurnal peak concentrations are not known and other compounds may also have reserve function.

Relationship between root/shoot ratio and sugar concentrations

The modelling results indicated that P-deficient plants accumulated more carbohydrate reserves because demand for assimilates in growing shoot organs (tillers and leaves) was reduced. This might also explain why root *v.* shoot DW partitioning was enhanced in these plants, and we therefore compared sugar concentrations in various compartments with root/shoot DW ratios (RSR) (Fig. 7). Across P treatments,

Table 2. Absolute root DW and sugar (glucose, fructose, sucrose and starch) content per plant at 30 DAT for IR64 and Azucena rice under abundant P supply (P+) and deficiency (P-)

SE, standard error of mean of three replications

| | IR64 | | | | Azucena | | | |
|---|-------|-------|-------|-------|---------|-------|-------|-------|
| | P+ | | P- | | P+ | | P- | |
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Root DW (g plant ⁻¹) | 0.290 | 0.032 | 0.247 | 0.014 | 0.312 | 0.010 | 0.260 | 0.019 |
| Root DW (% of plant DW) | 21.5 | – | 32.4 | – | 20.1 | – | 31.9 | – |
| Root sugar content (mg plant ⁻¹) | 7.97 | 1.31 | 10.59 | 1.24 | 17.04 | 1.22 | 16.46 | 2.05 |
| Root sugar content (% of plant sugar content) | 7.5 | – | 15.8 | – | 10.2 | – | 17.0 | – |

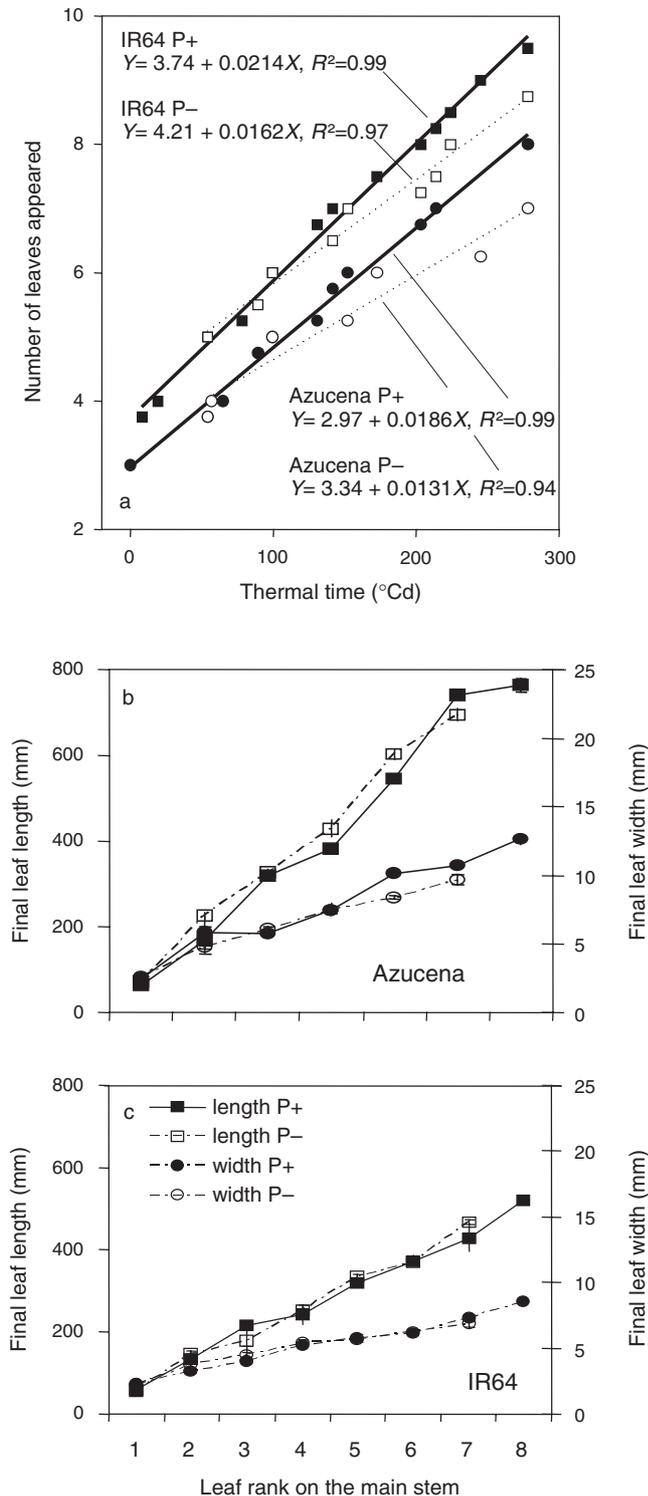


Fig. 3. (a) Number of leaves appearing on the main stem as a function of thermal time, for Azucena and IR64 rice with abundant P supply (P+) and deficient P supply (P-). (b, c) Fully expanded (ligulated) leaf blade length (left axis) and width (right axis) as a function of chronological leaf position on the main stem, for Azucena (b) and IR64 (c). Vertical bars indicate standard error of means of four replications.

a negative correlation was observed between leaf blade glucose concentration and RSR, and a positive correlation was observed between sheath reserve (starch + glucose) concentration and RSR. These relationships, which were similar for the two genotypes, showed an increasing slope as the plants developed, and were significant ($P < 0.05$) only for the later sampling dates (25 and 30 DAT).

Discussion

Mechanism of P deficiency effects on morphology seen through the model

On the basis of the concept of inter-organ competition for assimilates realised in the model (which is in part hypothetical) and the simulation results using the fitted model, a coherent theory of P deficiency effects on growth and development processes at the scale of the whole plant emerges. It strongly supports the recent finding that under P deficiency, assimilate resources are abundant in rice (Wissuwa *et al.* 2005). We suggest that P deficiency reduces demand for carbon in the leaves (through inhibition of leaf appearance rate, leaf extension rate and tiller production). Lower demand for assimilates in growing leaves is associated with reduced glucose concentrations in the blades, which can be seen as an indicator of smaller sink activity (Black *et al.* 1995; Yang *et al.* 2003; Roitsch and Gonzales 2004). The release of glucose from sucrose through cell wall invertase (CIN) activity in juvenile tissues is a rate-limiting step for growth (Black *et al.* 1995; Roitsch *et al.* 2000). The predicted increase of assimilate storage pools, known to be predominantly located in leaf sheaths (Luquet *et al.* 2005a), is supported by measurements. In contrast, no increase in glucose concentration was observed in roots, although their growth was stimulated by P deficiency (at least, relative to shoot growth), whereas sucrose concentration in roots increased significantly.

These observations suggest that root growth was not stimulated in terms of increased sink activity (which would probably be associated with an increase in glucose concentration), but more passively by the spill-over of excess assimilates from the shoot to the root. The increase of assimilate storage in sheaths and the build-up of sucrose in the roots support this hypothesis. In fact, Burleigh and Harrison (1999) and Shane *et al.* (2003) suggested that P deficiency is sensed in shoot organs and may only indirectly affect root growth behaviour.

The available data and hypotheses now enable us to propose a comprehensive theory of how the various morphological and phenological effects of P efficiency may come about and interact at the whole-plant level (Fig. 8). Phosphorus deficiency is sensed in the leaves when concentration drops below a critical value (Burleigh and Harrison 1999; Shane *et al.* 2003). This triggers at least three physiological responses induced by an unknown signal,

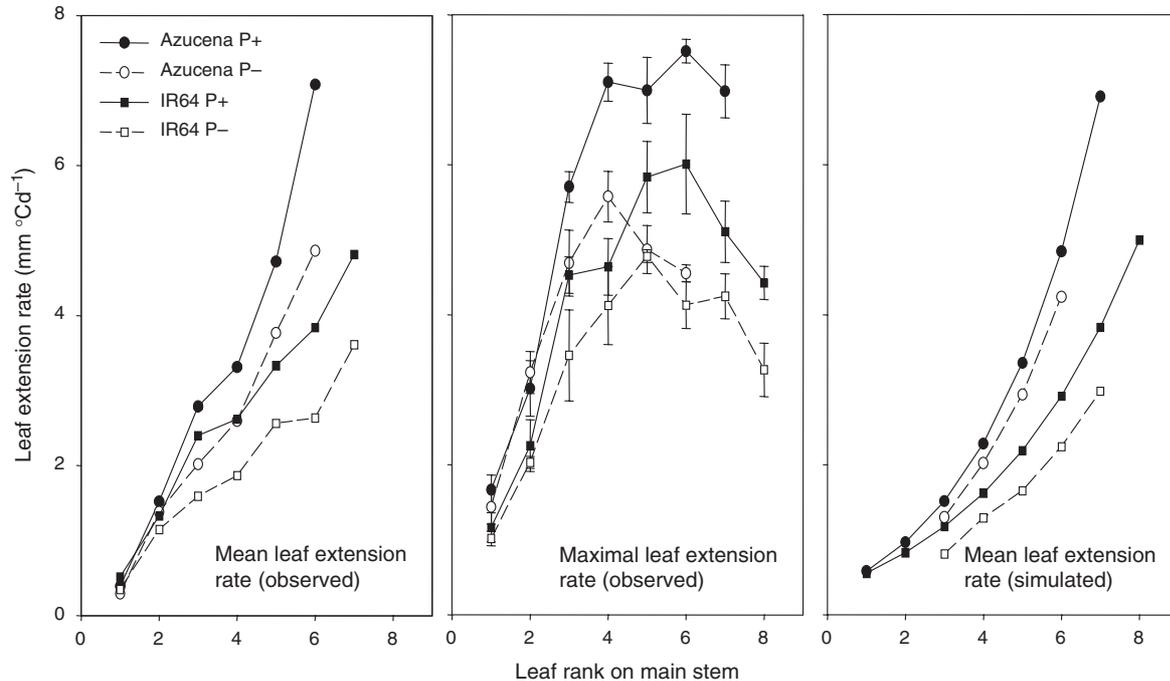


Fig. 4. Extension rates of leaf blades on the main stem as a function of leaf position (in chronological order), genotype (Azucena or IR64) and P treatment (deficient or adequate). Left: mean leaf extension rate computed from final leaf length and the corresponding, presumed duration of extension (tip appearance to ligule appearance). Centre: maximal leaf extension rate computed from daily observation on the length of the visible (exserted) part of the leaf blade. Right: simulated, mean leaf extension rates.

which may be hormonal (Burleigh and Harrison 1999) and may involve sugar signalling (Roitsch *et al.* 2000). These responses are (i) a decrease of the sensitivity of tillering to assimilate availability, (ii) a decrease in leaf extension rates and a proportional increase in leaf extension duration, while final leaf size remains unaffected, and (iii) the stimulation of citrate and phosphatase secretion by roots (Raghothama 1999; Lim *et al.* 2003; Shane *et al.* 2003; not measured in this study).

Tiller production of rice depends on the one hand on available buds (topological sites, equivalent to leaf axils; Hanada 1993; Tivet *et al.* 2001) and on the other hand on available resources (Dingkuhn 1996; Dingkuhn *et al.* 1999). Bud number usually does not limit tiller production. This was also the case in this study (data not presented), and the longer phyllochron observed under P deficiency can therefore not explain the reduction in tillering. In fact, excess production of assimilates observed in this treatment should theoretically favour tiller production. The opposite was the case, indicating that tillering was more specifically inhibited by P deficiency.

We hypothesise that the presumed, direct effects of P deficiency on leaf extension rates and tillering caused some other, more indirect effects. The increased extension duration of leaf blades, associated with a delay in ligule appearance, may be responsible for the longer plastochron observed under P deficiency. In rice, ligule appearance from the

previous leaf's sheath generally coincides with cessation of leaf blade extension and the appearance of the next leaf tip (Nemoto *et al.* 1995). In fact, Fournier *et al.* (2003) and Bos and Neuteboom (1998) showed for wheat that ligule appearance is the trigger for both events, enabling a coordinated morphogenesis of the culm. It is therefore likely that leaf extension duration, which depends on extension rate, feeds back on leaf appearance rate (which is equal to phyllochron⁻¹). However, the opposite causalities can be hypothesised as well, namely, that plastochron (leaf initiation rate⁻¹) governs phyllochron that governs leaf extension duration. Further studies are necessary to determine whether P deficiency directly affects leaf extension rate and duration, or phyllochron, or both.

The stimulation of root growth relative to shoot growth under P deficiency may also be an indirect effect, caused by under-utilisation of assimilates in above-ground organs. The accumulation of sucrose and starch in the leaf sheaths, generally considered a storage organ, clearly indicates that supply exceeded demand. Although leaf blade sucrose content remained remarkably constant among sampling dates and P treatments, glucose (Fig. 5) and fructose, which are cleavage products of sucrose and occurred at a ratio of nearly 1 : 1 in the shoot (data not presented), decreased sharply under P deficiency. We interpret this as an indication of reduced sink activity. CIN is considered a key enzyme for the regulation of sink activity of growing tissues (Black *et al.* 1995;

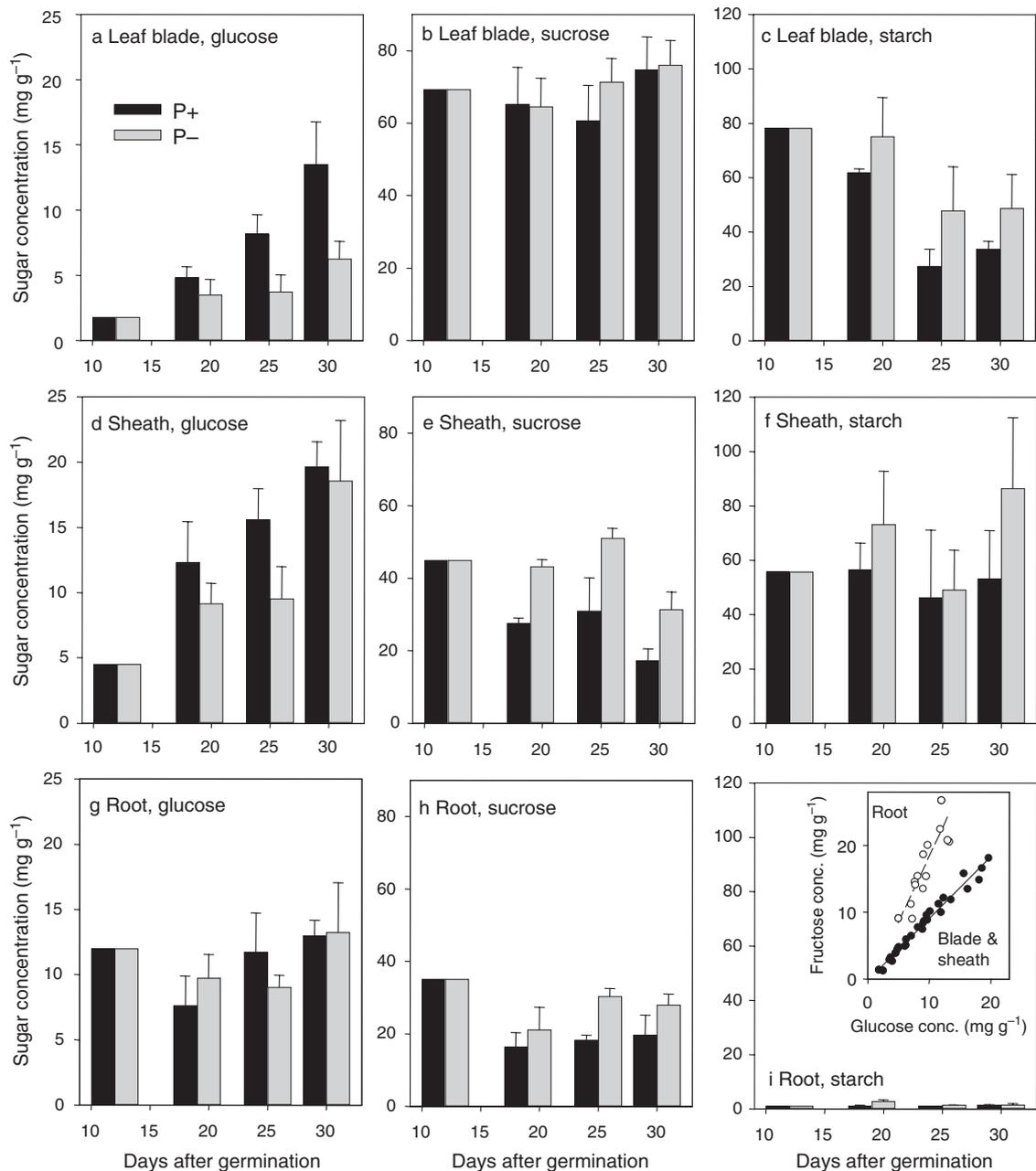


Fig. 5. Bulk organ concentrations of glucose (*a, d, g*), sucrose (*b, e, h*) and starch (*c, f, i*) in blades (*a, b, c*), sheaths (*d, e, f*) and roots (*g, h, i*), observed on Azucena rice under abundant (P+) and deficient (P-) phosphorus supply, on four consecutive sampling dates. Vertical error bars indicate standard error of three replicates. Inset: linear regressions between fructose and glucose concentrations in roots ($Y = -1.36 + 1.97X$, $R^2 = 0.78$) and shoot organs (blade or sheath; $Y = -0.25 + 0.93X$, $R^2 = 0.98$), for all dates, treatments and the two cultivars confounded.

Yang *et al.* 2003) and may have been down-regulated in shoot organs by the stress. In drought stressed rice, for example, the reduced expression of organ-specific cell wall invertase genes is associated with growth inhibition (Ji *et al.* 2005).

If local hexose concentration is indeed an indicator of growth related sink activity, the sinks in the root system

were not stimulated by P deficiency. In fact, root growth did not increase in absolute terms, but only relative to the shoot. But the substantial increase in sucrose concentration in the root system under P deficiency suggests that more sucrose was transported to the roots. Consequently, growth of the root system was probably not stimulated by signals, but simply benefited from greater assimilate supply. This

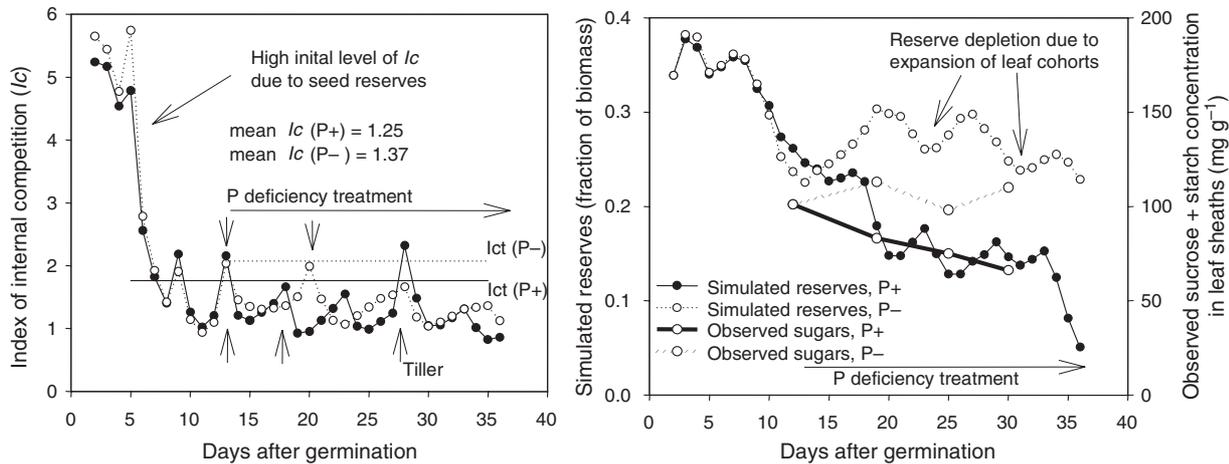


Fig. 6. Simulated kinetics of index of internal competition (I_c) and shoot carbohydrate reserves (fraction of dry matter) for Azucena rice under differential P supply (P+, P-). Left: tillers (\uparrow) are initiated when I_c exceeds the physiological threshold I_{ct} (horizontal line), which is higher under P deficiency (P-). I_c is, on average, higher under P- (1.37) than under P+ (1.25). Right: simulated reserves decrease over time, following a pattern similar to that of the observed sugar concentrations in the sheath. Oscillations are caused by organogenetic cycles. Results for IR64 rice were similar. Observed sucrose and starch reserves in the leaf sheaths are plotted for comparison.

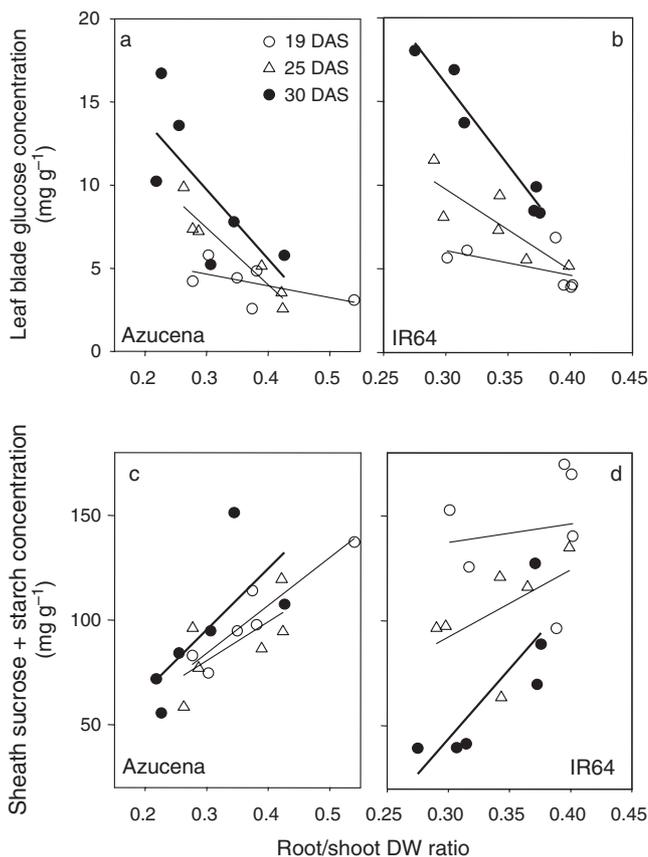


Fig. 7. Relationships between bulk organ sugar concentrations on DW basis (a, b: glucose in leaf blades; c, d: starch and sucrose in sheaths) and the root/shoot dry weight ratio at three sampling dates for Azucena (a, c) and IR64 (b, d) rice. Three replications and two P treatments confounded.

can be modelled as a spill-over of abundant resources from the shoot to the root, causing a modified biomass partitioning pattern.

Differences between genotypes seen through the model

Although P deficiency decreased dry matter growth by nearly 60% in both cultivars (Fig. 1), radiation efficiency (RUE) was reduced by only 13% in IR64 and 6% in Azucena (Table 1). Both cultivars had the same RUE in the control treatment. This indicates that dry matter production can be extremely variable even for a given light environment and given RUE, depending on how rapidly and efficiently assimilates are re-invested in leaf area production (Luquet *et al.* 2006).

The two cultivars differed strongly in morphology, Azucena having larger leaves, longer phyllochron and fewer tillers than IR64. Accordingly, the genotypes differ in the MGR , $PLAS$ and I_{ct} parameters, but not in the RUE_{pot} and RSR_{dem} parameters (Table 1, control columns). Under P deficiency, some of these parameters change little (RUE_{pot} and MGR), but others increase strongly ($PLAS$, I_{ct} and RSR_{dem}). As discussed in the previous section, the increase in RSR_{dem} , stronger for Azucena (+58%) than for IR64 (+35%) (Table 1), can actually be explained with a spill-over of excess assimilates into root growth, suggesting that RSR_{dem} , and thus root demand for assimilates, was not truly affected by P deficiency. This also explains why the model apparently over-estimated the transitory reserve pool while simulating its dynamics and treatment effects quite well (Fig. 6).

The P deficiency effect on $PLAS$ was greater in Azucena (+38%) than in IR64 (+23%), and its effect on I_{ct} was greater in IR64 than in Azucena (+88 and 18%, respectively,

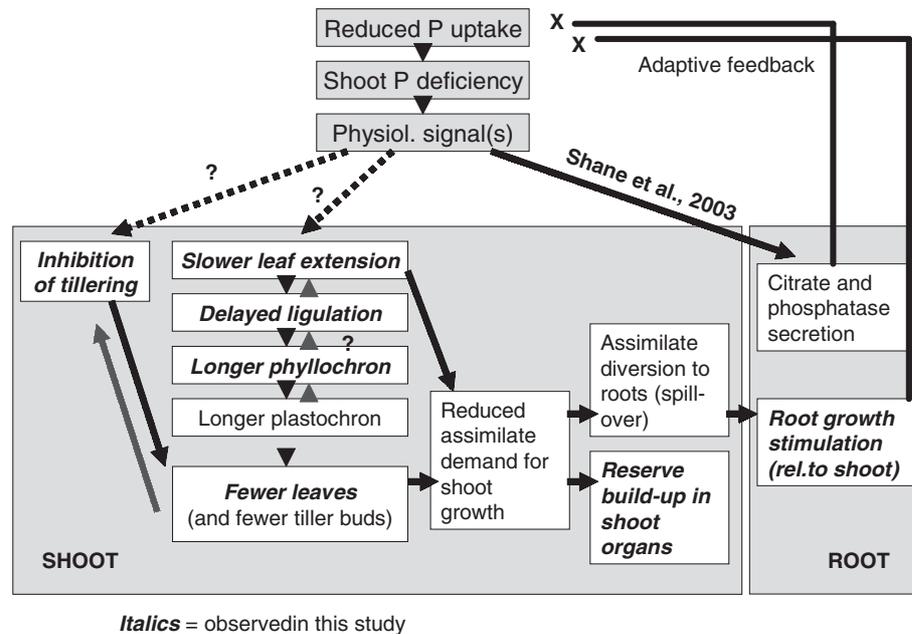


Fig. 8. Hypothetical diagram integrating various morphological and physiological effects of P deficiency in rice.

Table 1). These stress-induced changes in parameter values indicate that the parameters, which can be considered traits in the context of genotype comparison or phenotyping, are probably subject to $G \times E$ interactions. More studies are needed to evaluate the robustness of the model's crop parameters. In general, it can be expected that they are more stable (less prone to $G \times E$) than direct observations on plants, such as plant biomass, tiller number or leaf area at a given date and situation. But stresses, such as severe P deficiency, can obviously change plant reaction norms, necessarily involving changes in model parameter values. Such heuristic analyses (Hammer *et al.* 2002; Dingkuhn *et al.* 2005) can help distinguish constitutive and inducible, phenotypic plasticity. Constitutive plasticity translates environment variability into phenotype variability using constant reaction norms (or constant crop parameter values), and inducible plasticity is the result of changing reaction norms (or changing parameter values). However, as the example of root/shoot partitioning showed (change in root sink activity or spill-over of shoot assimilates?), the distinction between constitutive and inducible plasticity depends in part on model structure, and can thus be artificial. Proof of concept can only be obtained by relating genetic parameters, such as QTLs or candidate-gene polymorphisms, to model parameters and their variability. Such analyses are currently in progress.

Conclusion

Morphological and developmental effects of P deficiency were analysed for two contrasting rice varieties during

vegetative growth, in order to test the *EcoMeristem* model's capacity to capture genotypic differences and stress induced, phenotypic plasticity. The model proved to be of great value in relating to each other the various morphological and phenological changes the stress induced. According to *EcoMeristem*, two direct effects of the stress (inhibition of tillering and leaf blade extension rate) can sufficiently explain the remaining stress effects observed (reduction of biomass growth, longer plastochron, increased assimilate partitioning to roots, increase in plant carbohydrate reserves). The model also enabled measurement determining genotype-specific parameter values, a heuristic application. These might be of future use in model assisted phenotyping for process-based traits, which are difficult to estimate without the help of models.

Acknowledgment

The authors thank the Generation Challenge GCP of the CGIAR for partially funding this research.

References

- Black CC, Loboda T, Chen JQ, Sung SJS (1995) Can sucrose cleavage enzymes serve as markers for sink strength and is sucrose a signal molecule during plant sink development. In 'Proceedings of the first international symposium on sucrose metabolism.' (Eds HG Pontis, GL Salerno, EJ Echeverria) pp. 49–64. (American Society of Plant Physiologists: Rockland, MD)
- Boote KJ, Jones JW, Batchelor WD, Nafziger ED, Myers O (2003) Genetic coefficients in the CROPGRO-soybean model: links to field performance and genomics. *Agronomy Journal* **95**, 32–113.

- Bos HJ, Neuteboom JH (1998) Morphological analysis of leaf and tiller dynamics of wheat (*Triticum aestivum* L.): responses to temperature and light intensity. *Annals of Botany* **81**, 131–139. doi: 10.1006/anbo.1997.0531
- Brancourt-Hulmel M, Lecomte C (2003) Effect of environmental variations on genotype × environment interaction of winter wheat. *Crop Science* **43**, 608–617.
- Burleigh SH, Harrison MJ (1999) The down-regulation of MT4-like genes by phosphate occurs systematically and involves phosphate translocation to the shoots. *Plant Physiology* **119**, 241–248. doi: 10.1104/pp.119.1.241
- Chapman SC, Hammer GL, Cooper DW (2002) Linking biophysical and genetics models to integrate physiology, molecular biology and plant breeding (Chapter 12). In 'Quantitative genetic, genomics and plant breeding'. (Ed. MS Kang) pp. 167–187. (CAB International: Wallingford, UK)
- Clerget B, Dingkuhn M, Chantreau J, Hemberger J, Louarn G, Vaxsmann M (2004) Does panicle initiation in tropical sorghum depend on day-to-day change in photoperiod? *Field Crops Research* **88**, 21–27. doi: 10.1016/j.fcr.2003.11.008
- Dingkuhn M (1996) Modelling concepts for the phenotypic plasticity of dry matter and nitrogen partitioning in rice. *Agricultural Systems* **52**, 383–397. doi: 10.1016/0308-521X(95)00078-J
- Dingkuhn M, Johnson DE, Sow A, Audebert AY (1999) Relationships between upland rice canopy characteristics and weed competitiveness. *Field Crops Research* **61**, 79–95. doi: 10.1016/S0378-4290(98)00152-X
- Dingkuhn M, Luquet D, Quilot B, Reffye PD (2005) Environmental and genetic control of morphogenesis in crops: towards models simulating phenotypic plasticity. *Australian Journal of Agricultural Research* **56**, 1289–1302.
- Fournier C, Andrieu B, Ljutovac S, Saint-Jean S (2003) ADEL Wheat: a 3D architectural model of wheat development. In 'Proceedings of PMA03: plant growth modelling and applications. Beijing, China'. (Eds B-G Hu, M Jaeger) pp. 53–63. (Tsinghua University Press and Springer: Beijing)
- Hammer GL, Kropff MJ, Sinclair TR, Porter JR (2002) Future contributions of crop modelling — from heuristics and supporting decision making to understanding genetic regulation and aiding crop improvement. *European Journal of Agronomy* **18**, 15–31. doi: 10.1016/S1161-0301(02)00093-X
- Hanada K (1993) Chapter 4. Tillers. In 'Science of the rice plant'. pp. 222–258. (Food and Agriculture Policy Research Center: Tokyo)
- Hoogenboom G, White JW (2003) Improving physiological assumptions of simulation models by using gene-based approaches. *Agronomy Journal* **95**, 82–113.
- IRRI (2005) Rice knowledge bank. International Rice Research Institute, Los Banos, Philippines. Available at www.knowledgebank.irri.org [Verified 19 January 2006].
- Ji XM, Raveendran M, Oane R, Ismail A, Lafitte R, Bruskievich R, Cheng SH, Bennett J (2005) Tissue-specific expression and drought responsiveness of cell-wall invertase genes of rice at flowering. *Plant Molecular Biology* **59**, 945–964.
- Jitla DS, Rogers GS, Seneweera SP, Basra AS, Oldfield RJ, Conroy JP (1997) Accelerated early growth of rice at elevated CO₂: is it related to developmental changes in shoot apex? *Plant Physiology* **115**, 15–22.
- Kiniry JR, McCauley G, Xie Y, Arnold JG (2001) Rice parameters describing crop performance of four U.S. cultivars. *Agronomy Journal* **93**, 1354–1361.
- Lim JH, Chung IM, Ryu SS, Park MR, Yun SJ (2003) Differential responses of rice acid phosphatase activities and isoforms to phosphorus deprivation. *Journal of Biochemistry and Molecular Biology* **30**, 597–602.
- Luquet D, Zhang B, Dingkuhn M, Dexet A, Clément-Vidal A (2005a) Phenotypic plasticity of rice seedlings: case of phosphorus deficiency. *Plant Production Science* **8**, 145–151. doi: 10.1626/pp.8.145
- Luquet D, Zhang B, Dingkuhn M, Kim HK, Dexet A, Clément-Vidal A (2005b) Root/shoot phenotypic plasticity of rice seedlings: case of 5 genotypes under phosphorus deficiency. In 'Proceedings of the 4th international crop science conference'. Brisbane, Australia. Available at www.cropsscience.org.au [Verified 19 January 2006].
- Luquet D, Dingkuhn M, Kim HK, Tambour L, Clément-Vidal A (2006) *EcoMeristem*, a model of morphogenesis and competition among sinks in rice. 1. Concept, validation and sensitivity analysis. *Functional Plant Biology* **33**, 309–323. doi: 10.1017/FP05266
- Marschner H, Vetterlein D (1989) 'Fertilizer effect on root growth and drought resistance.' (University of Hohenheim, Plant Nutrition Institute: Hohenheim, Germany)
- Ming F, Mi G, Zhang F, Zhu L (2002) Differential response of rice plants to low-phosphorus stress and its physiological adaptive mechanism. *Journal of Plant Nutrition* **25**, 1213–1224.
- Nemoto K, Morita S, Baba T (1995) Shoot and root development in rice related to the phyllochron. *Crop Science* **35**, 24–29.
- Raghothama KG (1999) Phosphate acquisition. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 665–693. doi: 10.1146/annurev.arplant.50.1.665
- Reich PB (2001) Root–shoot relations: optimality in acclimation and adaptation or the “emperor’s new clothes”? In 'Plant roots, the hidden half'. (Eds Y Weisel, A Eshel, U Kafkaf) pp. 205–220. (Marcel Dekker: New York)
- Reymond M, Muller B, Leonardi A, Charcosset A, Tardieu F (2003) Combining quantitative trait loci analysis and an ecophysiological model to analyse the genetic variability of the responses of maize leaf growth to temperature and water deficit. *Plant Physiology* **131**, 664–675. doi: 10.1104/pp.013839
- Reymond M, Muller B, Tardieu F (2004) Dealing with the genotype × environment interaction via a modelling approach: a comparison of QTLs of maize leaf length or width with QTLs of model parameters. *Journal of Experimental Botany* **55**, 2461–2472. doi: 10.1093/jxb/erh200
- Roitsch T, Gonzales M (2004) Function and regulation of plant invertases: sweet sensations. *Trends in Plant Science* **9**, 606–613. doi: 10.1016/j.tplants.2004.10.009
- Roitsch T, Ehne R, Goetz M, Hause B, Hofmann M, Krishna Sinha A (2000) Regulation and function of extracellular invertase from higher plants in relation to assimilate partitioning, stress responses and sugar signalling. *Australian Journal of Plant Physiology* **27**, 815–825.
- Shane MW, Vos MD, Roock SD, Lambers H (2003) Shoot P status regulates cluster-root growth and citrate exudation in *Lupinus albus* grown with a divided root system. *Plant, Cell & Environment* **26**, 265–273. doi: 10.1046/j.1365-3040.2003.00957.x
- Tivet F, Pinheiro BDS, Dingkuhn M (2001) Leaf blade dimensions of rice (*Oryza sativa* L. and *Oryza glaberrima* Steud.). Relationships between tillers and the main stem. *Annals of Botany* **88**, 507–511. doi: 10.1006/anbo.2001.1447
- Wissuwa M (2003) How do plants achieve tolerance to phosphorus deficiency? Small causes and big effects. *Plant Physiology* **133**, 1947–1958. doi: 10.1104/pp.103.029306
- Wissuwa M, Ae N (2001) Genotypic variation for tolerance to phosphorus deficiency in rice and the potential for its exploitation in rice improvement. *Plant Breeding* **120**, 43–48. doi: 10.1046/j.1439-0523.2001.00561.x

- Wissuwa M, Gamat G, Ismail AM (2005) Is root growth under phosphorus deficiency affected by source or sink limitations? *Journal of Experimental Botany* **56**, 1943–1950. doi: 10.1093/jxb/eri189
- Wright SD, McConnaughay KDM (2002) Interpreting phenotypic plasticity: the importance of ontogeny. *Plant Species Biology* **17**, 119–131. doi: 10.1046/j.1442-1984.2002.00082.x
- Yang J, Zhang J, Wang Z, Zhu Q, Liu L (2003) Activities of enzymes involved in sucrose-to-starch metabolism in rice grain subjected to water stress during grain filling. *Field Crops Research* **81**, 69–81. doi: 10.1016/S0378-4290(02)00214-9
- Yin X, Struik PC, Kropff MJ (2004) Role of crop physiology in predicting gene-to-phenotype relationships. *Trends in Plant Science* **9**, 426–432. doi: 10.1016/j.tplants.2004.07.007

Manuscript received 31 October 2005, accepted 18 January 2006

THE N STORES IN TREES: AN OVERVIEW

By Marie-Odile JORDAN

RU 1115 Plantes et systèmes de culture horticoles, INRA, Avignon, France.

Introduction

Trees are dependent on their nitrogen (and carbon) reserves to ensure regrowth after vegetative accidents (burning, diseases) or pruning, for winter survival and for early-spring growth. Most of the studies attempted to relate reserve formation and mobilization to (i) climate: photoperiod (Coleman et al. 1991, Bollmark et al. 1999) or temperature (Arora et al. 1992, Arora and Wisniewski 1994, Bollmark et al. 1999), (ii) soil N availability (Bollmark et al. 1999, Neilsen et al. 2001), (iii) phenological stage: overwintering (Sauter et al., 1996; Rowland and Arora, 1997), or (iv) growth, which was the most investigated controlling factor. Growth could partly explain the yearly dynamic of the stores. In autumn, after cessation of shoot elongation, most of the N uptake is incorporated into the stores (Millard 1996; Tagliavini *et al.* 1997). In early spring, mobilisation in was related to biomass production (Deng *et al.* 1989, Coleman et al. 1993, Neilsen *et al.* 1997, Hartmann 1975).

However, the amounts of N involved in storage and remobilisation remained difficult to evaluate accurately according to plant culture conditions. Thus, the N stores are not evenly distributed within the crown, which makes the quantification impossible with classical sampling methods. The portions of the woody structure chosen for analyses have to be selected on an architectural basis. From this viewpoint, the organs above ground could be described as an organised system of axes, each of them being composed of a chronological sequence of phytomers. The phytomers are elementary units constituted of a node, an internode, a leaf and an axillary bud (White 1979). Analyses performed at phytomer levels allowed to establish a mapping of N concentration gradients within the woody structure, to identify the zones that are most affected by a modification of the soil N availability, and to relate locally N content and growth. Thus, shoot growth results from phytomer emergence (apical meristem plastochronal activity), phytomer growth (internode and leaf elongation, thickening) and phytomer ramification (development of the axillary bud into a rosette or an axis, depending whether or not the internodes are elongated) (Crabbé 1987). Similarly, reproductive growth can be described as flowering, fruit set and thickening.

We intend here to develop this approach on young peach trees (*Prunus persica* L. Batch). The seasonal dynamics of the stores will first be described a tree level, in relation with C metabolism. The ability of the reserves to be mobilized at any time of the year in response to a trophic stress will also be tested. The N reserves, however could not be identified by their biochemical form, since they comprised proteins like rubisco or aquaporines which are involved in other plant metabolisms, like C assimilation or water stress regulation. The determinations were therefore focused on total- and protein-N, whose variations were usually assumed to be indicative of those of the stores. Thus, N represented less than 2% of the total dry matter (0.5% for protein-N) and varied strongly ($\pm 400\%$ for total-N) throughout the year.

Experimental design: a short description

Experiment 1: Forty 1-year-old peach rootstocks (*Prunus persica*, cv "GF 305") were cultivated on hydroponic conditions. The experimental design was composed of 4 sets of 10 culture tanks (148x24x12 cm), each of them being related to a 1000l container located below ground. The nutrient solution is pumped (delivering rate: 200l h⁻¹) from the container to the end of each culture line, and returns by gravity to the container, within 10mn. Each 3h, sampling and analyses of the solution is performed on the return way. The initial pH (5.5) and NO₃

concentrations (0.1mM) were restored by injections of nitric acid and solution enriched in $\text{Ca}(\text{NO}_3)_2$. The depletion within the 3h corresponded to the net N uptake of the trees. The volume of the solution is also readjusted to compensate transpiration. The whole procedure: nutrient control and data recording, is automated. A severe pruning, which removed 78% of the total biomass, was applied on August 29 on all trees.

Experiments 2 and 3: One-year-old rootstocks (30 and 70 resp.) were grafted with pushing buds of peach (cv "RO52" on cv "GF 305") in March then transplanted in 20l pots and raised outside. Water (2l day^{-1}) and nutrients (solution with a concentration of 1g l^{-1} of a commercial 14/7/27 % NPK fertiliser) were provided without restrictions. In autumn, a deprivation treatment was applied from the end of shoot growth (when all leaves were fully expended) to leaf fall, *i.e.* from mid-September to mid-November. For experiment 2, nitrate and other nutrients were provided 3 times a week (Monday, Wednesday and Friday) by 300 cm^3 nutrient solution, containing, according to the treatment 0, 1.5, 3, 4.5 or $6\text{ g NO}_3\text{ dm}^{-3}$ as $\text{Ca}(\text{NO}_3)_2$. These treatments, applied to 6 trees, were designated 0N, 1N, 2N, 3N and 4N respectively. For experiment 3, only the 1N and 3N solutions were used for 35 trees each. In both cases, the field capacity was restored in the pots by automatic irrigation with tap water on Tuesday, Thursday, Saturday and Sunday. The following spring, from bud burst (February) to harvest, all trees received a solution labelled with 1.5% ^{15}N , having a NO_3 concentration of 1.5meq l^{-1} . The supply was adjusted so as to maintain some solution available to the plants throughout the day, in saucers placed under the pots. Fertilisation was superior to growth needs (*i.e.* to the amounts of N incorporated in the current year's shoots and roots). Destructive harvests of three individuals per treatment were taken on May 29 and June 13 for experiment 1, and distributed throughout the store constitution and mobilisation periods for experiment 2: September 17, October 2, 16 and 31 and November 22 in autumn, February 14 (bud swelling), March 1, 11 and 28 (first visible petals, full blossoming, and fruit set/beginning of stem elongation) and May 16 (end of the first growth flush) in spring.

The trees were described before application of the treatments by counting the number of phytomers of each main and secondary axis. That allowed ranking the phytomers, which constituted each axis, in order of occurrence from the base (or insertion point) of the axis up to the last phytomer visible on the apex. The position of each secondary axis was noted using the rank of its father phytomer: *i.e.* the rank of the main axis phytomer to which belongs the axillary bud that developed and elongated. Three corresponding classes of secondary axes were defined according to their position on the main axis, *i.e.*: basal from 1 to 12, median from 13 to 27 and upper from 28 to the top. The whole trees were harvested in experiment 2, but sampling was restricted to 8 positions in experiment 3. A basal, a median and an upper part of the main axis; a basal, a median and an upper short (having less than 15 phytomers) secondary axis, a basal and a median long (having between 28 and 36 phytomers) secondary axis were taken. For the secondary axes sampling was restricted to phytomers 1 to 7. All samples were immediately immersed in liquid nitrogen, and deep-frozen for biochemical determination (Médiène *et al.* 2002).

For experiment 2, the position of each vegetative bud was recorded in spring, and its development followed once a week. Each of them grew either into a rosette of a few leaves or, when the internodes elongated, into a proleptic -or new- axis. The number of expanded leaves, which almost corresponded to the number of phytomers, was counted. The transformation of a rosette into an elongated axis and indeed into a ramified axis was noted, and the number of ramifications (current year sylleptic axes) of each proleptic axis counted. The total length and the diameter were measured at harvest.

Results

Evidence of short-term reserves- Figure 1 gives the mean hourly N uptake per tree of experiment 1 before and after pruning. From August 25 to 29, N uptake was typically related to solar radiation. It increased during the day and decreased during the night or when the trees are shaded. Pruning stopped N uptake within 1 hour. Absorption remained nil until September 14, then increased slowly day per day. At September 24, the mean values were about one third of what was observed before pruning. During the meantime however regrowth started: new vegetative buds were emitted around September 4 (picture 1), the leaf of the rosette began to expand around September 7 (picture 2) and the new axes elongation was significant at September 13 (picture 3). These development stages were performed exclusively at the expenses of the N reserves since they occurred before restoration of root

activity.

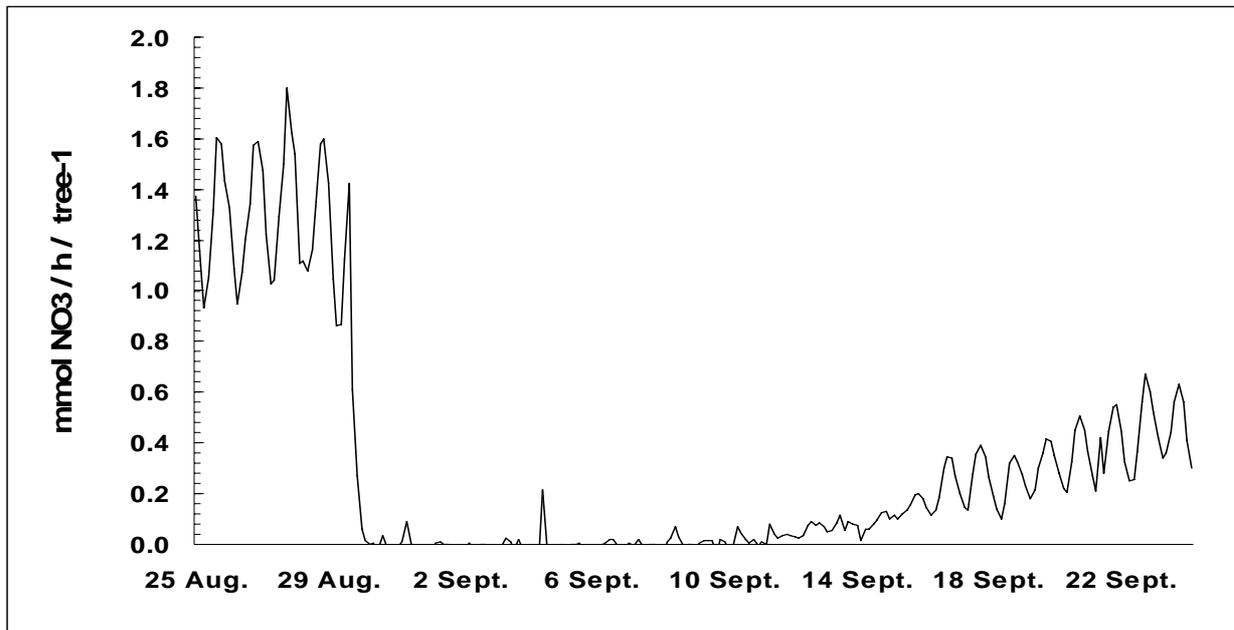


Figure 1- Mean N uptake in $\text{mmol NO}_3 \text{ h}^{-1} \text{ tree}^{-1}$ from August 25 to September 22. Trees were cultivated in hydroponic conditions without N limitation, and severely pruned (removing of 78% of the total biomass) at August 29.

Picture 1: September 4



Picture 2: September 7



Picture 3: September 13



Pictures 1 to 3 - September 4: Emission of new vegetative buds on the 10 cm long parent shoot (picture 1); **September 7:** Leaf expansion, i.e. formation of the initial rosette (picture 2); **September 13:** New axes elongation, i.e. plastochronal activity in the apical meristem (picture 3).

Quantification of the N stores- Figure 2 indicates the amounts of ^{14}N , i.e. of the N accumulated in the trees of Experiment 2 before winter rest, in relation to fall N treatment. Total N content increased from 1.29 to 3.13g with fertilisation up to a threshold value that corresponded to the 2N supply. N

uptake and assimilation is then limited, either by plant metabolism or by culture conditions, and depended no more on soil availability. The following spring, most of this old N (from 0.53 to 2.25 g) was incorporated into the current year shoots and fruits during the first flush of growth. At harvest, the depletion of the perennial structure was similar for all trees, whatever the treatment: they contained $0.87 \pm 0.11\text{g }^{14}\text{N}$. At harvest, the biomass of the new shoots and fruits was strongly correlated (R^2 significant at 0.1% level) to N mobilisation.

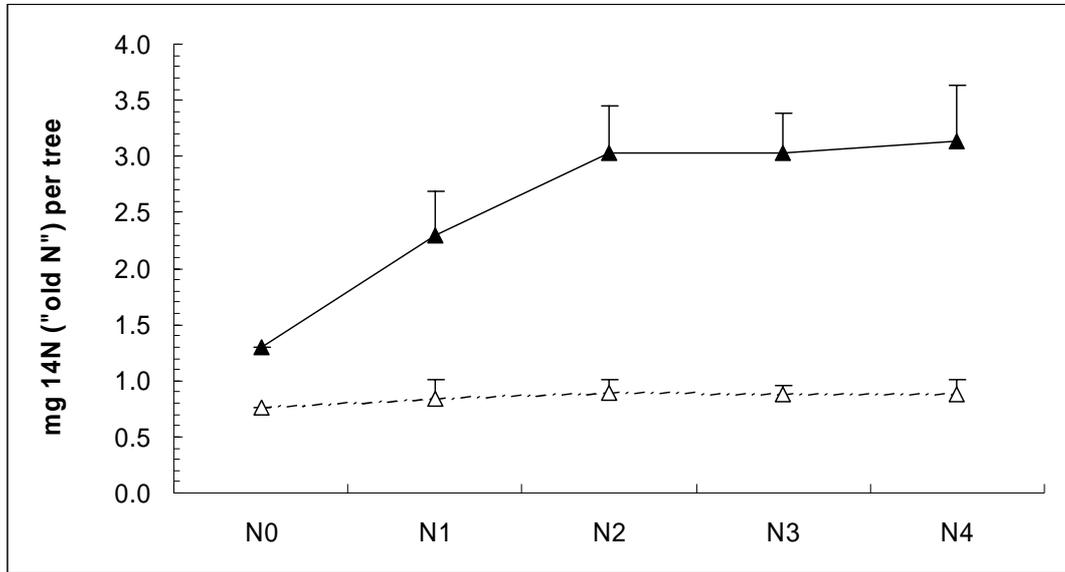
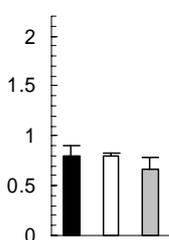


Figure 2- Effect of autumn N supply on the tree ^{14}N contents (in g). Full line and full triangles: whole trees, broken lines and open triangles: perennial structure (old wood and roots). Each point represented the mean value and the standard deviation of the 6 replicates harvested on May 29 (for 3 trees) and June 13 (for 3 trees) in experiment 2.

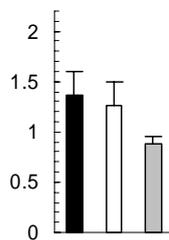
N allocation within the crown: N was not evenly distributed within the crown as demonstrated figure 3 for the trees of Experiment 3, harvested at November 22, *i.e.* at the end of the N accumulation period. For the non-limited control trees, *i.e.* the trees of the 3N treatment, the total-N concentrations (black bars) varied from 0.47 to 1.78 %, according (i) to the order, (ii) to the length and (ii) to the position of the axes. They increased from the base to the top of the crown for the main axes (or trunks). This gradient reversed for the short secondary axes, and no changes could be evidenced for the long secondary axes. At a given position, the concentrations were the lowest in the main axes and the highest in the short secondary axes. The effect of a deprivation treatment (white bars) was limited to the basal and median secondary axes, *i.e.* to the organs, which exhibited the highest N contents. The decrease was about 50% for the long axes and 30% for the short axes.

At leaf fall, the N pool was mainly constituted of soluble proteins (grey bars), which represented 84%, 63% and 45% of the total-N in the trunks, in the upper and median secondary axes, and in the basal secondary axes respectively. This proportion was not affected by axes length.

Trunk upper part



Upper short second order axis



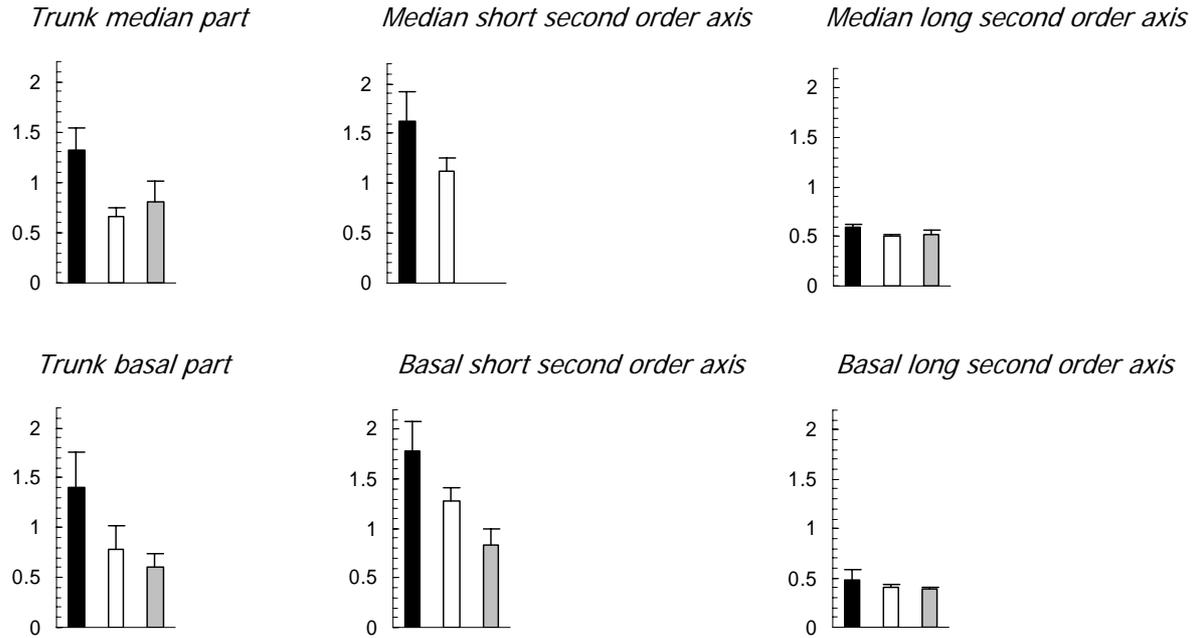
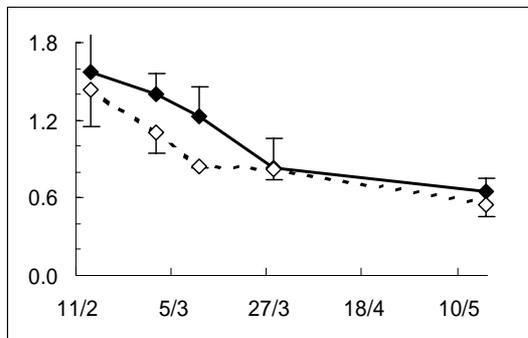


Figure 3- Total-N and soluble protein-N mean concentrations in $g\ 100g\ dry\ matter^{-1}$, at different parts of the crown. *s*Trees of Experiment 3, harvest of November 22, means *s* and *s*standard deviation). Black bars: total-N in control trees (3N treatment), white bars: total-N in deprived trees (1N treatment), grey bars: soluble protein-N in control trees.

Mobilisation patterns in relation with C metabolism: Between November 22, *i.e.* beginning of winter rest, and February 14, *i.e.* bud swelling, no differences in total- and proteins-N concentrations could be observed in the tree crown. N mobilisation (figure 4) began at burst (February 14) and was achieved at fruit set (March 28). During that period, the long median secondary axes of the control and the deprived trees lost respectively 63% and 42% of their N. Depletion at fruit set was, in terms of concentration, similar for all trees whatever their treatment: $0.51g\ N\ 100g\ dry\ matter^{-1}$. At that date, the contribution of the C stores (non-structural-carbon: TNC and starch) to early spring metabolism was also achieved. C mobilisation was however immediately followed by an important accumulation of starch. Thus, at May 16 (end of the first growth flush), the starch and TNC concentrations were higher as at bud swelling. It depended moreover on the previous autumn treatment, which was likely induced by difference of shoot growth, and consequently in photosynthesis abilities.

Total-N concentrations ($g\ 100g^{-1}$)



TNC and starch concentrations ($g\ 100g^{-1}$)

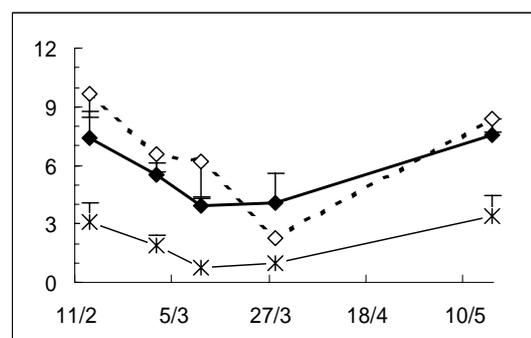
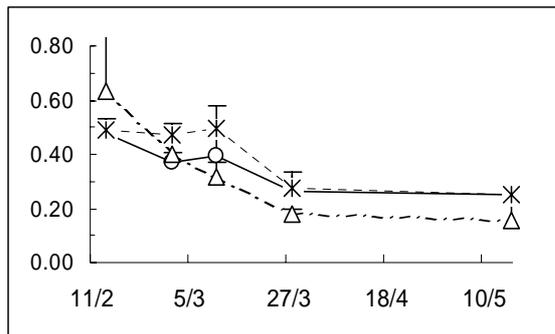


Figure 4- Spring mobilisation of total-N, non-structural-carbon (TNC) and starch in $g\ 100g\ dry\ matter^{-1}$, in the long median secondary axes. Separate determinations were made for wood and bark and the mean concentrations of each axis were recalculated according to their respective weights. Each point represented the mean value and the standard deviation of 3 trees of Experiment 3, harvested from February 14 to May 16. Full

lines: control trees (3N treatment), broken lines: deprived trees (1N treatment), dots: total-N, squares: non-structural-Carbon, stars: starch.

N mobilisation varied strongly among the axes (figure 5). The total-N concentrations were the lowest in the median parts of the trunk and the highest in the median short secondary axes, whatever the observation date. It seemed that the axes, which contributed the less to N storage, were the most depleted (in terms of concentration) in spring. In the trunks, the N pool is mainly composed of soluble proteins (concentration: $0.64 \text{ g } 100\text{g}^{-1}$), which degraded rapidly and regularly from February 14 to Mach 28, from bud swelling to fruit set. In the secondary axes, the protein-N concentrations were lower at burst (short axes: 0.49 , long axes: $0.47 \text{ g } 100\text{g}^{-1}$) and decreased significantly only after blossoming.

Total-N concentrations ($\text{g } 100\text{g}^{-1}$)



Soluble protein-N concentrations ($\text{g } 100\text{g}^{-1}$)

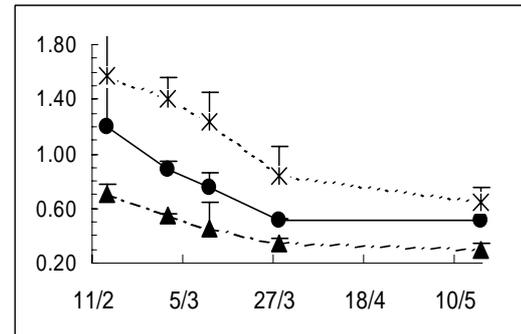


Figure 5- Spring mobilisation of total, and soluble protein-N in the median parts of the trunk, in the median long and short secondary axes (Controls of Experiment 3: means and standard deviations. Full lines - dots: median long secondary axes, broken lines - triangles: median trunks parts, dotted lines - stars: median short secondary axes, open symbols: soluble protein-N; full symbols: total-N.

Relation with shoot growth evaluated on an architectural basis, results from Experiment 2: Almost 32% of the phytomers of the perennial structure developed a new vegetative bud in spring, whatever the treatment. Once emitted, these new bud could either develop into a rosette of a few leaves, or into a new proleptic axis. N treatment affected (i) the number of expanded leaves of the buds that stayed at rosette stage, and (ii) the proportion of buds which transformed into new axes (or elongation rate). It had no effect on the further growth (diameter, length of the stems, number of expanded leaves) of those new proleptic axes, which depended exclusively on their position in the woody structure. It should be noticed that the new shoots began to elongate around fruit set, *i.e.* when N mobilisation was achieved.

These effects were however not evenly distributed within the crown. Only the median and the upper secondary parent axes were affected by treatment. On these positions the elongation rate increased from 28 to 100% with fall N fertilisation. These variations of the number of growing axes allowed adjusting shoot biomass production and N mobilisation. Thus, the number of leaves of the rosette represented less than 2% of the shoot biomass at harvest. Despite the fact that the results were obtained on 2 separated experiments (2 and 3), the concentration gradients of total N could explain the variations with position of new shoots growth (number of leaves and length) during the first growth flush.

Conclusion

N stores play a major role on shoot development in spring or after a vegetative accident like pruning. Shoot growth in early spring is quantitatively correlated to N mobilisation. It could be assumed to

stores are entirely emptied before fruit set, since the amounts of N which remained in the perennial structure was small and not affected by treatment.

N deposition and further spring growth varied with the positions, the lengths and the orders of the axes of the woody structure. Therefore N availability modified not only tree size but also tree shape: fertilisation increased the density of the proleptic axes in the median and upper part of the trees.

Several questions had now to be solved. Little is known about the contribution of the current year N uptake. It is classically assumed that it become significant only after the first growth flush, when carbon assimilation was sufficient to ensure the growth needs and the root metabolism. The relation between C and N metabolisms needs also further investigations.

References

- Arora, R., M.E. Wisniewski and R. Scorza. 1992. Cold acclimation in genetically related (sibbling) deciduous and evergreen peach (*Prunus persica* L. Batsch). I. Seasonal changes in cold hardiness and polypeptides of bark and xylem tissues. *Plant Physiol.* 99:1562-1568.
- Arora, R. and M.E. Wisniewski. 1994. Cold acclimation in genetically related (sibbling) deciduous and evergreen peach (*Prunus persica* L. Batsch). II. A 60-kilodalton bark protein in cold-acclimated tissues of peach is heat stable and related to the dehydrin family of proteins. *Plant Physiol.* 105:95-101.
- Bollmark, L., L. Sennerby-Forsse and T. Ericsson. 1999. Seasonal dynamics and effects of nitrogen and carbohydrate reserves in cutting-derived *Salix viminalis* plants. *Can. J. For. Res.* 29:85-94.
- Coleman, G.D., T.H.H. Chen, S.G. Ernst and L. Fuchigami. 1991. Photoperiod control of poplar bark storage protein accumulation. *Plant Physiol.* 96:686-692.
- Coleman, G.D., J.M. Englert, T.H.H. Chen T.H.H. T.H. Fuchigami. 1993. Physiological and environmental requirements for poplar (*Populus deltoides*) bark storage degradation. *Plant Physiol.* 102:53-59.
- Crabbé JJ. 1987. Aspects particuliers de la morphogenèse caulinaire des végétaux ligneux et introduction à leur étude quantitative. IRSIA, Brussels. 116p.
- Hartmann, W. 1975. Die Reservekohlenhydratgehalt in einjährigen Trieben von *Juglans regia* L. und seine Veränderung während der Winterruhe. *Angew. Bot.* 49:237-251.
- Médiène, S., L. Pagès, M-O. Jordan, J. Le Bot and S. Adamowicz. 2002. Influence of nitrogen availability on shoot development in young peach trees (*Prunus persica* (L.) Batsch). *Trees*, 16: 547-554.
- Neilsen, D., P. Millard, G.H. Nielsen and E.J. Hogue. 1997. Sources of N for leaf growth in a high-density apple (*Malus domestica*) orchard irrigated with ammonium nitrate solution. *Tree Physiology* 17:733-739.
- Neilsen, D., P. Millard P., G.H. Nielsen and E.J. Hogue. 2001. Nitrogen uptake, efficiency of use, and partitioning for growth in young apple trees. *J. am. Soc. hort. Sci.* 126:144-150.
- Millard P. 1996. Ecophysiology of the internal cycling of nitrogen for tree growth. *Z. Pflanzenernahr. Bodenk.* 159: 1-10. Proctor, J.T.A., R.L. Watson and J.J. Landsberg. 1976. The carbon budget of a young apple tree. *J. am. Soc. hortic. Sc.* 101:579-582.
- Rowland, L.J. and R. Arora. 1997. Proteins related to endodormancy (rest) in woody perennials. *Plant Sci.* 126:119-144.
- Sauter, J.J., M.E. Wisniewski M.E. and Witt. 1996. Interrelationships between ultrastructure, sugar levels, and

frost hardiness of ray parenchyma cells during frost acclimation and deacclimation in poplar (*Populus canadensis* Moench 'robusta') wood. *J. Plant Physiol.* 149:451-461.

Tagliavini M, Quartieri M, Millard P. 1997. Remobilised nitrogen and root uptake of nitrate for spring leaf growth, flowers and developing fruits of pear (*Pyrus communis* L.) trees. *Plant Soil* 195: 137-142.

Wetzel, S., C. Demmers and J.S. Greenwood. 1989. Seasonally fluctuating bark proteins are a potential form of nitrogen storage in three temperate hardwoods. *Planta* 178:275-281.



Annexes

Physico-Chemical Changes in the Fruits of Two Coconut (*Cocos nucifera* L.) Hybrids during Ripening. A NIRS-boosted Study

Fabrice Davrieux, Alexia Prades and Isabelle Mialet-Serra

CIRAD, TA80/16 73 rue J.F. BRETON 34398 Montpellier Cedex 5 FRANCE

Stéphane Dupuis

Universités d'Aix-Marseille I et II, Faculté des Sciences de Luminy, Marseille FRANCE

Rebecca Assa

Université de Cocody et Centre National de la Recherche Agronomique, Station Marc DELORME, Port-Bouët, Abidjan, IVORY COAST

Keywords: Perennial crop, quality, harvest, sample selection, calibration, sustainable research methodology.

Abstract

Near infrared spectroscopy was used to assess total soluble sugar (TSS) and lipid contents in the freeze-dried kernel of coconuts from two cultivars: PB121 or MYD x WAT from Ivory Coast and VRD x VTT from Vanuatu. The spectra of 385 samples were acquired on a NIRSystems 6500 monochromator. Using this spectral library, a classification algorithm was applied to extract 128 samples representative of the library. The latter were used to construct calibration equations in reference to HPLC laboratory analyses for sugar contents and automatic extraction by organic solvent for lipid contents. The TSS and lipid contents predicted by the model provided information on coconuts quality from the two cultivars studied at different stages of ripeness. This study demonstrated the suitability of a near infrared spectroscopy tool for assessing the quality of coconut palm fruits. The methodology chosen was shown to be relevant for the research and production constraints encountered in the coconut commodity chain. The cost of the study was lowered by 70%, whilst the volume of organic solvents was reduced by more than 90%.

INTRODUCTION

Copra oil (from dried coconut kernel), which ranks seventh in the world oils and fats trade, is a major outlet for coconut growing (FAOSTAT, 2004), but it is not the only one. Over the last few years, new coconut-based products like canned coconut milk have been appearing on the markets in producing countries and are gradually establishing a foothold on the European market. For these outlets, a coconut variety is not required to provide tonnes of copra per hectare, but tonnes of fresh kernel (Ranasinghe, 1996). For instance, new criteria for assessing and selecting coconut varieties are appearing (Baudoin and Rouzière, 1996) and need to be measured. As a corollary, analytical methods adapted to products and production contexts have to be developed.

The coconut palm is a perennial plant whose continuous production throughout the year undergoes climatic variations. The most common varieties are diploid and cross-fertilizing, which leads to substantial variability in the phenotypic traits of the fruit. Monitoring a single variety over a period of three years generates around 350 kernel's samples. Given the number of varieties to be assessed, the structure of the farms (Kullaya and Sangare, 1996) and the uncertainty of the coconut world market (Voituriez, 2000), it is clear that proposing a methodology making it possible to reduce the number of

laboratory analyses whilst using inexpensive, versatile and robust tools (Quinsac and Ribaillet, 1998) would be advantageous.

Near infrared spectroscopy (NIRS) is one of the methods that satisfied our operating conditions. It does not require much equipment in the field and can be used to monitor several fruit quality criteria. This study set out to test the ability of NIRS to assess the different coconut cultivars according to predefined quality criteria. It also set out to develop a sustainable research methodology, in adequation with the coconut production context.

MATERIAL AND METHODS

Harvesting and Sample Preparation

The study involved two coconut hybrids: PB121 from Ivory Coast (Nucé de Lamothe and Bénard, 1985), cultivated worldwide, which is a cross between the Malayan Yellow Dwarf and the West African Tall (MYD x WAT) and the Vanuatu hybrid which is a cross between the Vanuatu Red Dwarf and the Vanuatu Tall (VRD x VTT) (Labouisse et al., 2005). Six palms were selected per study plot for each of the cultivars. Bunches of increasing ripeness were harvested from the six palms. In the coconut palm, the degree of ripeness is determined by the rank number of the frond subtending the fruit bunch. Rank 15, the most immature fruit stage in our study, corresponded to 4-month-old nuts and rank 24 to mature coconuts (12 to 13 months), harvested for copra preparation. There was an interval of around one month between two successive ranks.

Three nuts were taken from each of the harvested bunches. A 50 g sample of kernel was taken from each nut and freeze-dried. Three hundred and eighty five kernel samples, which were representative of the different stages of ripeness, were harvested in Vanuatu (246) and Ivory Coast (139) during the 2001 to 2004 period. The freeze-dried samples were transported to the laboratory and ground in liquid nitrogen using an IKA-Werke (Germany) A10 M20 type grinder equipped with blades, until a particle size of 200 µm was obtained.

Biochemical Analyses

1. Determination of Lipid Contents. Lipids were extracted from the freeze-dried kernels using an automatic extractor: ASE[®] 200 (Accelerated Solvent Extraction, DIONEX Inc., USA). After homogenization of the thawed samples, a 2 g sample aliquot was taken and placed in the extraction unit along with 2 g of Fontainebleau sand. The extraction solvent was petroleum ether at 60°C. The flush was set at 100% and the number of cycles at 5, with a static time of 7 min. The lipid content of the sample was the ratio between the weight of fatty matter extracted and the weight of the test-piece. It was expressed in g of lipids per 100 g of dry matter (% db).

2. Determination of Total Soluble Sugar Content. Sugars were extracted on the same apparatus: ASE[®] 200. Extraction was carried out immediately after lipid extraction on the meal remaining in the extraction unit. The solvent used was an 80% ethanol solution at 60°C. There were 5 cycles, a static time of 7 min, and a flush of 100%. The sugar extracts recovered were diluted and filtered. They were injected into a DIONEX-DX 600 high performance liquid chromatograph fitted with a CarboPac MA-1 column. The eluant was 612.10^{-3} mol sodium hydroxide at a flow rate of 0.4 ml.min⁻¹. After separation, sugars were detected by a Dionex ED50 pulsed amperometric system. The total sugar content corresponded to the sum of the contents of the different carbohydrate compounds detected

in the kernel and was expressed in g of sugars per 100 g of dry matter (% db).

Spectral Analyses

NIRS acquisitions were carried out in mini-cups on a Foss-Perstorp 6500 analyser (FOSS NIRSystems Inc., USA) with a spinning module equipped with an automatic sampler taking up to 50 cups. The spectral data were collected and processed by WinISI version 1.5 (Infrasoft International, USA).

Three grams of ground kernel were analysed by diffuse reflection from 400 to 2500 nm at 2 nm intervals. For each sample, a sequence of 32/32 measurements (32 measurements of the reference ceramic, then 32 measurements of the sample) was performed. The sample measurements were averaged to obtain the absorbance spectrum in $\log(1/R)$ where 1 was the reflection of the ceramic and R the reflection of the sample for each wavelength.

Statistical Analyses

The descriptive statistics of the reference and NIRS predicted data were computed using STATISTICA 6.1 Software (StatSoft Inc., USA).

Relevant information was extracted from the matrix of spectral data by Principal Components Analysis (PCA). Based on the PC matrix, the Mahalanobis H distance from the average spectrum was calculated for each spectrum. An H distance over 3 for a given sample corresponded to a probability of less than 0.01 that the sample belonged to the population. Sample selection was based on this distance (Shenk and Westerhaus, 1991).

Partial least squares or PLS regression was applied to establish mathematical models between the spectral and chemical data (Prévot, 2004). The number of terms (factors) to be introduced was determined by cross-validation.

The efficiency of the models developed was estimated by statistical criteria, which reflected quality of fit and precision (Workman, 1992), such as the coefficient of determination (RSQ), the standard error of calibration (SEC), which represented the precision of the model depending on the number of terms introduced. The standard error of cross-validation (calculated through 4 sub-groups) was used to select the optimum number of PLS factors and to estimate the predictive error of the model (Wold, 1978).

The relationship between the standard deviation (SD) of the reference data and the SECV, expressed as the ratio of performance to deviation (RPD) is a useful criterion for the effectiveness of a calibration (Williams, 1993). Indeed, a calibration for which the RPD is over 3 can be used to predict a criterion with a precision approaching the reference analysis; a RPD between 2 and 3 corresponds to a model applicable to "rough" sample sorting.

RESULTS AND DISCUSSION

Spectral Analysis

Even though the spectral profiles of the two varieties/countries were identical, the absorbance levels were clearly different. It suggested that the average biochemical compositions of the two cultivars were similar in terms of constituent types; the same compounds were found throughout the ripening process and only the relative contents of those compounds varied from one cultivar/country pair to the next.

The graph of the standard deviations (Fig.1), calculated for each wavelength for all of the 385 samples, clearly confirmed that the major variations in the spectra were

located at the fats and/or cellulose bands. This result clearly reflected a spectral population that was representative of all stages of nut ripening. Indeed, as nuts ripen, the two major metabolic phenomena that occur are the synthesis of lipids, which make up the reserves of the seed, and construction of the kernel cell walls, which switches from a gelatinous to a solid state.

Principal Components Analysis

Three successive PCA were calculated, based on the second derivatives of the spectra for the wavelength segment between 908 nm and 2500 nm. This iterative procedure allowed to identify and eliminate 23 outliers samples ($H > 3$). Thirteen principal components were chosen and explained 99.7% of total variance. The first 4 PCs explained 52.9%, 37.0%, 5.1% and 2.5% respectively.

The cluster of points was uniformly distributed according to the first main plane, which explained 89.9% of the initial variance. There was no apparent group structure, be it according to cultivar types and/or countries. Superimposing the samples from Ivory Coast on the first two main planes of the PCA calculated using the samples from Vanuatu (Fig.2) clearly confirmed the choice of a single population for calibration development. In other words, the spectral variability of the samples from Ivory Coast was described by that of the Vanuatu samples.

Sample Selection

In order to save time and money, it was useful to be able to base selection on the spectra of the samples that were most representative of the entire population, so as only to carry out reference analyses on those samples. As a spectrum is the result of the elementary absorption of different constituents depending on their concentration, it is correct to imagine that once artefacts due to physical properties (particle size) and atypical spectra (oxidized, etc.) have been overcome, a spectrum is representative of its composition (types of constituents and concentration). Based on this, it was assumed that one sample was sufficient to represent its neighbourhood.

This approach was applied to the 362 samples kept, by fixing a neighbourhood global H distance of 0.6. The relatively small number (128) of selected samples, i.e. 35% of the whole, reflected relatively low variability in the library. Of the 128 samples, 96 were from Vanuatu and 32 from Ivory Coast.

Biochemical Analyses

The total soluble sugar (TSS) contents of the 128 samples were between 3.5 and 25.5% db, the lipid contents between 34.0 and 76.8% db (Tab.1).

Changes in the TSS and lipid contents of the kernel in the two hybrids was antagonistic (Fig.3), which argued in favour of an assumption whereby sugars contribute towards the synthesis of lipid reserves in the coconut (Rajagopal and Ramadasan, 1999). The highest sugar contents and lowest lipid contents corresponded to the most immature ranks: ranks 15 to 20 depending on the hybrids. The correlation between the lipid content and the TSS content showed a coefficient of determination of 0.61.

According to these initial analyses, the ripening processes of the two hybrids were different. The appearance of kernel in the nut, which was later in Ivory Coast, did not seem to affect lipid contents once ripe. Whilst the kernel of the Vanuatu hybrid seemed to stabilize at maximum lipid content as of rank 20, the kernel of the Ivorian hybrid did not reveal any plateau at the most evolved stages of the fruit.

NIRS Calibration

The value of the RPD coefficient for total sugars was equal to 5.1 (Tab.2). This value, like the SECV value (0.817%), corresponded to an efficient model for the prediction of total soluble sugar contents in the kernels. The coefficient of determination (RSQ) of the reference values depending on the predicted values (Fig.4) was equal to 0.983, the slope of the regression was 1.00 and bias was nil. In comparison, Tarkosova and Copikova, 2000 found a SECV of 1.04 and a RPD of 4.17 for total sugar content of Cavendish bananas.

The fit of the model for lipid contents was not efficient (RPD = 3.1 and SECV = 2.75), but the distribution of this component was highly dissymmetrical; 78% of samples had a lipid content over 55%. The RSQ of the regression (Fig.4) of the reference values depending on the predicted values fell from 0.92 to 0.65 if only those values over 55% were kept. The model developed to predict lipid contents could be applied to classify samples, e.g. by class of contents centred on 30%, 40%, 50%.

Analysis of the Predicted Values and Conclusions of the Study

As the calibration model was judged to fit well for total sugars, the contents of the remaining 234 samples were predicted. The average of the TSS contents for all the samples was $8.4 \pm 0.3\%$ db, that of the Vanuatu hybrid was 8.2 and 8.6 for PB121. For lipid content, only a sample screening was possible. The spectral library needs to be developed further, but the prediction indicators are heading in the right direction.

Using NIRS as a sample selection tool led to the laboratory analysis of 128 samples+23 samples removed from the spectral library after the first PCA, i.e. 151 samples in the whole. Laboratory analysis of 385 samples for the constituents described here would have taken 40 weeks in a so-called conventional study using standardized methods. With the combined use of near infrared spectroscopy tools and automatic extraction, it only took 10 weeks (Tab.3), which broke down into 9 weeks of chemical analyses and 4 days of spectroscopy to construct the spectral library. Moreover, the methodology used made it possible to use only 9 litres of solvents rather than 109 (Tab.3). Pollution level was thus reduced by 92%.

The duration, as long as the cost of the study (cost of solvents +cost of equipment, based on a 5 years linear amortization + labour, based on the French Ministry of Foreign Affairs 2003 technician tariff), was 70% less than a so-called conventional study. Time and cost were closely correlated since 97% of the overall cost consisted of labour costs.

Thus, this study demonstrated the feasibility of using near infrared spectroscopy to assess the quality of coconut fruits at different ripening stages. It also highlighted that implementing an analytical methodology combining automatic extraction methods and NIRS enables substantial savings to be made, whilst protecting the environment. The coconut commodity chain has a new tool at its disposal to assess the numerous and various cultivars, which would make it possible to improve the production management in terms of both quantity and quality. However, the full potential of this methodology and, in particular, the ability of NIRS instrument to work in coconut production context has still to be demonstrated.

Acknowledgements

The authors wish to thank the Directors and staff at the Vanuatu Agricultural Research and Training Centre (VARTC) and the Marc DELORME Station, Centre National de la Recherche Agronomique (CNRA) in Ivory Coast for their precious collaboration. The authors are grateful to Mr. G. Piombo and Mrs A. Clément for their technical support and advices.

Literature Cited

- Baudoin, L. and Rouzière, A. 1996. Search for traits usable in coconut breeding to promote multi-purpose uses and competitiveness of the coconut. Proc. Promoting multi-purpose uses and competitiveness of the coconut. Chumphon, Thailand 26-29 September. p.24-27.
- FAOSTAT data. 2004. www.fao.org
- Kullaya, K.A. and Sangare, A. 1996. La filière cocotier en Afrique : situation actuelle et perspectives. *Oléagineux*. 3:130-135.
- Labouisse, J.P., Sileye, T., Hamelin, C., Baudoin, L., Bourdeix, R., Rouzière, A. 2005. Coconut (*Cocos nucifera* L.) genetic improvement in Vanuatu: Overview of research achievements from 1962 to 2002. Part 2: Improvement of the Vanuatu Tall by hybridization, OCL (in press).
- Nucé de Lamothe, M. de and Bénard, G. 1985. L'hybride de cocotier PB-121 (ou MAWA) (NJM x GOA). *Oléagineux*. 40:261-266.
- Prévot, H. 2004. Comparaison de méthodes statistiques et neuronales pour l'établissement d'équations de calibrage en spectrométrie de réflexion diffuse dans le proche infrarouge. Thesis, Faculté universitaire des Sciences agronomiques de Gembloux, Belgique.
- Quinsac, A. and Ribaillet, D. 1998. Perfectionnements dans le contrôle de qualité des oléagineux. OCL. 5:284-287.
- Rajagopal, V. and Ramadasan, A. 1999. Advances in plant physiology and biochemistry of coconut palm. APCC, Jakarta.
- Ranasinghe, T.K.G. 1996. Marketable coconut products and summary of representative project profiles opportunity. Proc. Promoting multi-purpose uses and competitiveness of the coconut. Chumphon, Thailand 26-29 September. p.24-27.
- Shenk, J.S. and Westerhaus, M.O. 1991. Population definition, sample selection and calibration procedures for near infrared reflectance spectroscopy. *Crop Science*. 31: p.469-474.
- Tarkosova, J. and Copikova, J. 2000. Determination of carbohydrate content in bananas during ripening and storage by near infrared spectroscopy. *J. Near Infrared Spectrosc.* 8:p.21-26.
- Voituriez, T. 2000. Risk and uncertainty of world demand for palm, palm kernel and copra. OCL. 7:p.140-146.
- Williams, P. 1993. What does the raw material have to say? *NIR News*. 4:13.
- Wold, S. 1978. Cross-Validation Estimation of the Number of Components in Factor and Principal Components Models. *Technometrics*. 20:397-405.
- Workman, J.J. 1992. NIR Spectroscopy Calibration Basics. p. 247-280. In: D.A. Burns and E.W. Ciurczak (eds), *Handbook of Near-Infrared Analysis*, Vol. 13, Practical Spectroscopy Series. Marcel Dekker, New York.

Tables

Table 1: Descriptive statistics for the constituents of the 128 selected samples (% db)

| | TSS content | Lipid content |
|------------------------------|-------------|---------------|
| Mean | 9.0 | 59.3 |
| Standard deviation | 4.2 | 9.2 |
| Range | 3.5-25.5 | 34.0-76.8 |
| Standard Error of Laboratory | 0.2 | 0.4 |

Table 2: Calibration and validation results (% db)

| Constituent | N ¹ | Mean | SD ² | SEC ³ | RSQ ⁴ | SECV ⁵ | Nb PLS ⁶ | RPD ⁷ =SD/SECV |
|-------------|----------------|--------|-----------------|------------------|------------------|-------------------|---------------------|---------------------------|
| TSS | 119 | 8.824 | 4.138 | 0.572 | 0.981 | 0.817 | 10 | 5.1 |
| Lipid | 113 | 60.170 | 8.451 | 2.485 | 0.914 | 2.750 | 1 | 3.1 |

¹Number of samples kept by the model (test *t*) out of 128, ²standard deviation of the calibrating population, ³standard error of calibration, ⁴coefficient of determination, ⁵standard error of cross validation, ⁶Number of PLS terms, ⁷Ratio performance deviation

Table 3: Comparison of solvent volume, time and cost of the study according to the type of methodology

| Type of methodology | with NIRS with ASE [®] n=151 | with NIRS without ASE [®] n=151 | without NIRS with ASE [®] n=385 | without NIRS without ASE [®] n=385 |
|----------------------|---|--|--|---|
| Organic solvents (L) | 9 | 71 | 21 | 109 |
| % reduction | 92% | 35% | 81% | |
| Time (week) | 10 | 16 | 23 | 40 |
| % reduction | 75% | 60% | 43% | |
| Cost (euros) | 16613 | 24742 | 34234 | 54858 |
| % reduction | 70% | 55% | 38% | |

Figures

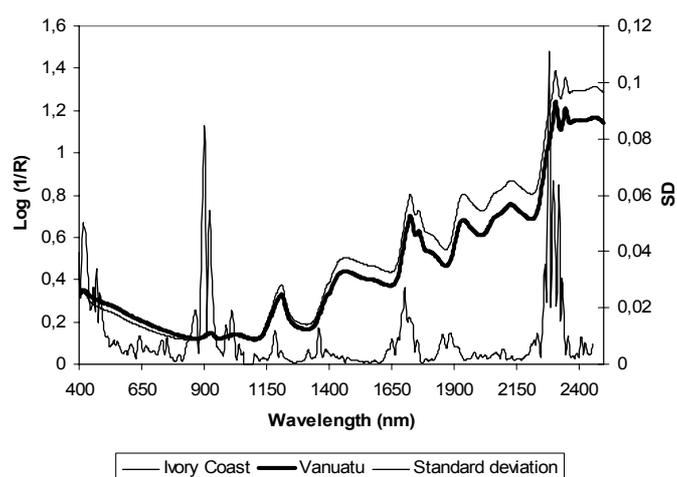


Fig. 1: Average spectra of freeze-dried kernels from the two coconut hybrids and standard deviation at each wavelength

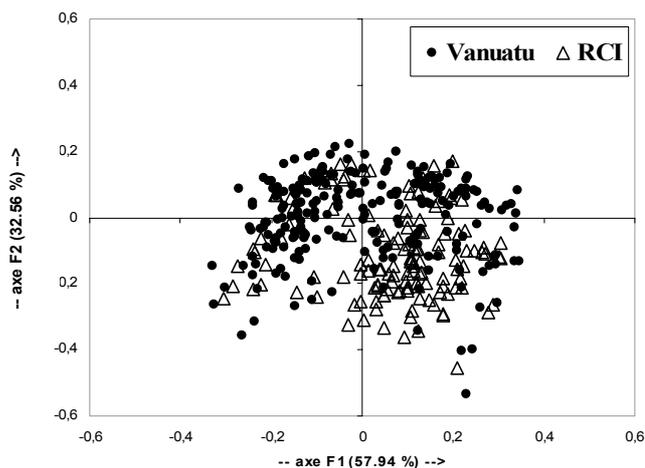


Fig. 2: Two-dimensional scatter plot of the 246 coconut kernel samples from Vanuatu (VTU) and 139 from Ivory Coast (RCI) for the first two PCs

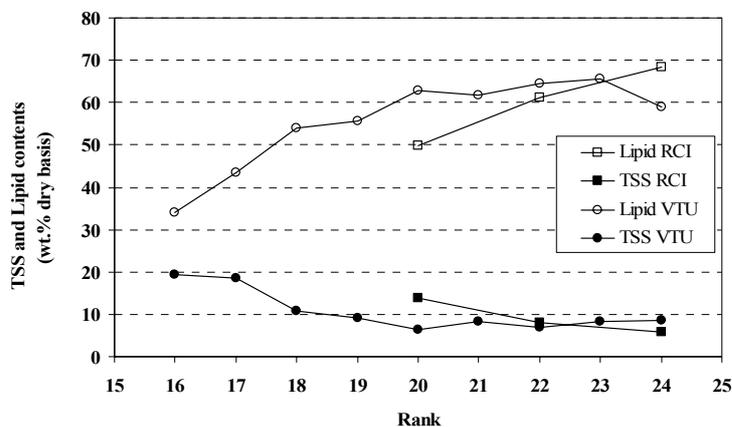


Fig. 3: Total soluble sugar and lipid contents (% db) of the 128 selected freeze-dried coconut kernels according to the ripening stage

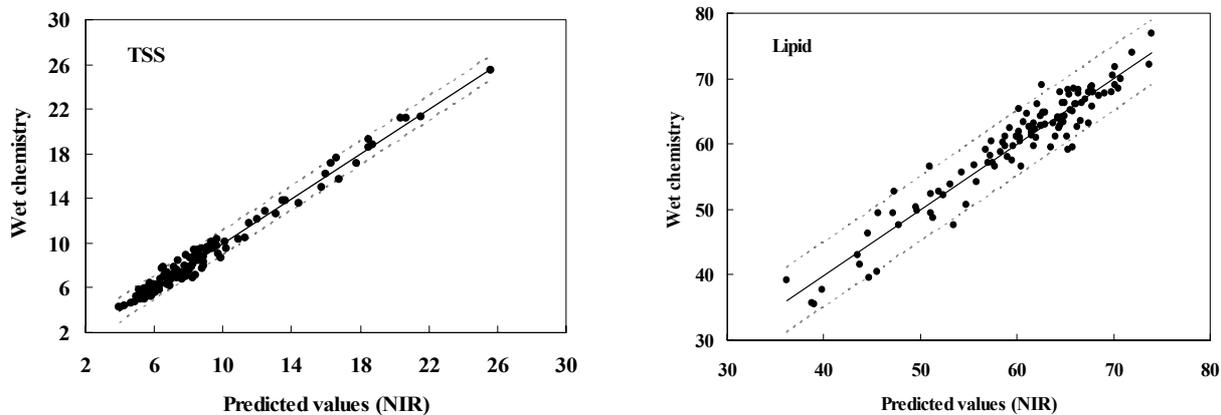


Fig. 4: Correlation between wet chemistry and NIR predicted values for total soluble sugar content and lipid content (Confidence interval 95%)

A virtual peach fruit model simulating changes in fruit quality during the final stage of fruit growth

FRANÇOISE LESCOURRET^{1,2} and MICHEL GÉNARD¹

¹ INRA, Plantes et Systèmes de culture Horticoles, Domaine Saint-Paul, site Agroparc, 84914 Avignon cedex 9, France

² Corresponding author (lescou@avignon.inra.fr)

Received September 24, 2004; accepted December 11, 2004; published online August 1, 2005

Summary A virtual fruit model simulating seasonal changes in several peach (*Prunus persica* (L.) Batsch) fruit quality traits during the final growth stage is presented. The quality traits considered are fruit size, the proportion of total fruit mass consisting of fruit flesh, dry matter content of the flesh and the concentrations of sucrose, glucose, fructose and sorbitol in the flesh, which are used to calculate a sweetness index. The virtual peach fruit model was developed by adapting and integrating three existing process-based models describing fruit dry mass growth, fruit fresh mass growth and sugar accumulation in the flesh into one complex system. Data sets of peach fruit growth and quality obtained from one field site over several years were used to estimate parameters and evaluate the virtual peach fruit model. Output from the model showed good agreement with the field data. Insight into the complex nature of the virtual peach fruit model, i.e., its ability to show emergent properties, was accomplished by conducting a series of theoretical experiments. The virtual peach fruit model was shown to be sensitive to management and environmental factors (leaf: fruit ratio, stem water potential and, to a lesser extent, weather). Its ability to generate simple laws relating to physiological variables and quality parameters was also demonstrated. Finally, the virtual peach fruit model was able to reveal complex behaviors resulting from changes in water potentials or leaf: fruit ratios over time.

Keywords: fructose, glucose, mass, sorbitol, sucrose, sugar concentration, system, theoretical experiment.

Introduction

In recent years, fruit quality has become an increasingly important aspect of fruit production. For example, in Europe, the new market organization enjoins farmers to form producer organizations whose goal is to improve fruit quality. Research efforts directed toward understanding the effects of climate and management techniques on fruit quality are needed, and mathematical models are useful frameworks for these research efforts (Sansavini 1997). Fruit quality, even when reduced to organoleptic qualities (such as sweetness or acidity) that meet consumer demand, is a multi-criterion concept. Each quality trait is the result of a complex chain of biological processes

that depend on environmental conditions. These processes are interrelated (e.g., sugar metabolism depends on carbon fluxes) and their effects on quality traits may be opposite (e.g., enhancing water fluxes into fruit increases fruit size but decreases sugar concentration; Génard and Lescourret 2004). Clearly, a useful fruit quality model must take into account several quality traits, the underlying processes and their interactions.

However, following the pioneering work of C.T. de Wit (see van Ittersum et al. 2003), most process-based fruit models have focused on carbon relationships leading to predictions of fruit growth in dry mass. Such photosynthesis-driven models have been developed for apples (Baumgaertner et al. 1984, Seem et al. 1986), grapes (Gutierrez et al. 1985), kiwifruit (Buwalda 1991), olives (Abdel-Razik 1989), peaches (Grossman and DeJong 1994) and tomatoes (Heuvelink and Bertin 1994). Some models have dealt with nitrogen content, representing nitrogen and carbon dynamics on a similar conceptual basis (sink-driven assimilation and allocation using priority rules; Wermelinger et al. 1991). Researchers have modeled water accumulation in fruit, considering water uptake and transpiration per unit fruit area as constant (Lee 1990) or variable (Génard and Huguet 1996). In a more mechanistic work applied to tomatoes, the difference between water potentials in the stem and the fruit was assumed to be the driving force of water import rate (Bussièrès 1994). Another tomato water model focused on the role of pedicel resistance and calyx transpiration (Bussièrès 2002). A few models of fruit metabolism describing synthesis and degradation processes have been designed for sugar (Génard and Souty 1996) and citric acid accumulation (Lobit et al. 2003). However, few models consider several processes together (except Bussièrès 1993, 1995 and Fishman and Génard 1998, with interrelated C and water transports).

In this paper, we propose a modeling approach that can simultaneously simulate changes in several quality traits throughout the final stage of fruit growth, using the example of peach (*Prunus persica* (L.) Batsch). These traits include: fruit size, the most important commercial criterion for growers; the proportion of the total mass consisting of fruit flesh, which is important for the consumer; dry matter content of the flesh; and flesh concentrations of various sugars. Our threefold approach

was based on the existence of specialized models (i.e., devoted to categories of traits) already published, validated and used. First, we integrated these models into a virtual peach fruit model to simulate the interactions between processes and their consequences on quality. Second, we validated the approach by comparing model predictions with data collected over different years and growing conditions. Third, we studied the emerging behavior of the virtual peach fruit model as a product of its complexity. By emerging behavior we mean, in the sense of systems theory, properties that cannot be reduced to those of the components (i.e., to the basic knowledge incorporated into the models). Three questions structured the study of emergent behavior: (1) To what extent is the virtual peach fruit model sensitive to environmental factors? (2) Is it able to generate simple and general laws? (3) Is it able to produce complex behaviors? To answer these questions, we performed theoretical experiments with the virtual peach fruit model, involving typical environmental factors of fruit crops. The factors were: weather, which cannot be controlled and is nowadays subjected to intensive modeling research (e.g., modeling projected effects of global change (Tubiello et al. 2002, Wolf 2002)); plant water status variables resulting from the regime of water uptake, which can be partially controlled; and leaf:fruit ratio, which can be strongly controlled by thinning practices.

Description of the virtual peach fruit model

The virtual peach fruit model (Figure 1) represents the quality buildup of a “mean” fruit on the fruit-bearing stem, which is the basic production unit for peach growers. This unit is a 1-year-old stem (20–50 cm long) that bears fruit and new shoots. The virtual peach fruit model runs on a daily time step. Peach fruit development occurs in three stages, with active growth during the first and third stages and stone hardening during the second (plateau) stage. The virtual peach fruit model concerns the third stage corresponding to the enlargement of flesh cells and includes 80% of fruit growth. The vir-

tual peach fruit model is a combination of three sub-models of which one describes the management of carbon, one the management of water and one the buildup of sugars. The sub-models, their adaptations and connections are described briefly below.

Carbon sub-model

The carbon sub-model has been described by Lescourret et al. (1998). It has been successfully tested with data sets from several field sites and years (Génard et al. 1998), and used to analyze fruit growth from different viewpoints (Génard et al. 1998, Quilot et al. 2002, Walcroft et al. 2004).

The fruit-bearing stem is divided into three compartments: fruits, 1-year-old stem and leafy shoots, considering carbon reserves (except in fruit) and dry mass in each. The pool of carbon assimilates available daily is the assimilation of leaves plus that mobilized from either local reserves, or from other parts of the tree (Walcroft et al. 2004). Here we consider only local assimilates. Light-saturated leaf photosynthesis, an important component of leaf photosynthesis, is possibly changed by: (1) a feedback inhibition through the leaf storage reserves; and (2) leaf water potential, as detailed by Ben Mimoun et al. (1999). Total daily leaf photosynthesis is the sum of hourly photosynthesis by sunlit and shaded leaves. Sunlit and shaded leaf areas are computed with coefficients describing the between-shoot shadow (originating from the foliage of the surrounding shoots) and the within-shoot shadow (mutual shadow of leaves within a shoot), respectively (input data). Such coefficients are assumed to follow a simple seasonal pattern of variation and may be estimated using gap fractions derived from fisheye photographs. Carbon assimilation by fruits is considered on a similar basis, with a temporal component accounting for the seasonal decline in fruit photosynthesis. If required, reserves are mobilized first from the leafy shoot, then from the 1-year-old stem. It is assumed that a fixed fraction of reserves can be mobilized each day.

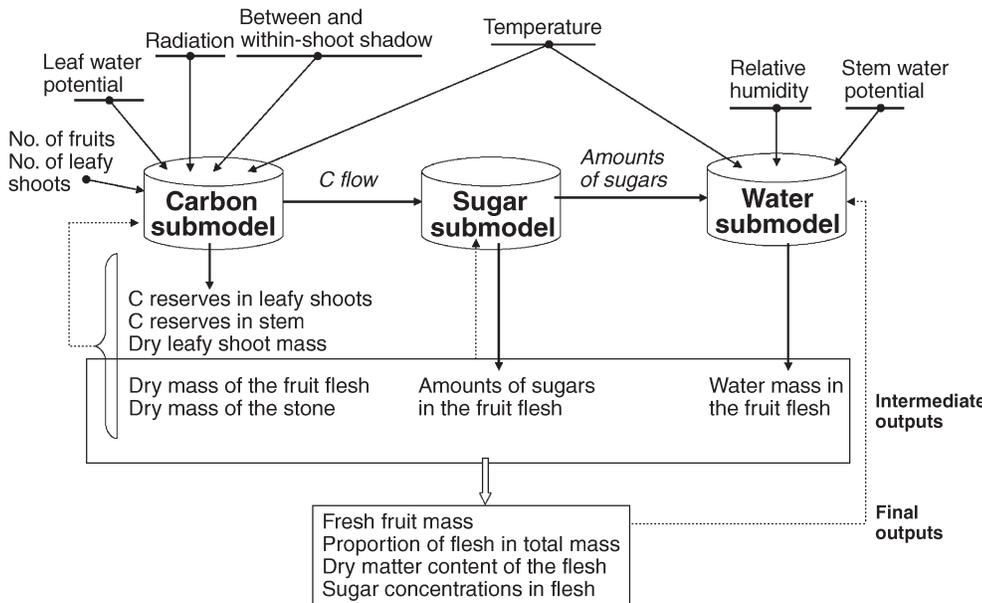


Figure 1. General scheme of the virtual peach fruit model. External variables are underlined, arrows indicate data flows and dashed lines represent feedback information.

Carbon is allocated according to organ demands (as modified by the effects of temperature and of dry mass already accumulated) and priority rules. Maintenance respiration costs are given first priority, with vegetative and reproductive growth given second and third priority, respectively. The assimilates unused for maintenance and growth accumulate in the reserve pools of leafy shoots and 1-year-old stem.

Daily carbon demand, D , for fruit growth is subjected to a new parameterization in the present work. An important component of D is $\Delta DM^{\text{pot}}/\Delta dd$, the potential growth rate of the individual fruit in terms of degree days (dd), modeled as:

$$\frac{\Delta DM^{\text{pot}}}{\Delta dd} = \text{RGR}^{\text{ini}} \text{DM} \left(1 - \frac{\text{DM}}{\text{DM}^{\text{max}}} \right) f(\text{dd}) \quad (1)$$

with:

$$f(\text{dd}) = \begin{cases} 1, & \text{if } \text{dd} < \text{dd}_{\text{min}} \\ \frac{\text{dd}_{\text{max}} - \text{dd}}{\text{dd}_{\text{max}} - \text{dd}_{\text{min}}}, & \text{if } \text{dd}_{\text{min}} < \text{dd} < \text{dd}_{\text{max}} \\ 0, & \text{if } \text{dd} > \text{dd}_{\text{max}} \end{cases}$$

where RGR^{ini} is the initial relative growth rate, DM^{max} is the limiting final mass, and dd_{min} and dd_{max} are parameters (in dd) corresponding to the beginning and end of fruit maturation, respectively.

Adaptation of the original model to calculate carbon inputs of the sugar sub-model (see next section) requires taking account of the sharing of carbon between the flesh and the stone within the fruit compartment. Deriving an empirical relationship between stone dry mass, DM_s , and total fruit dry mass, DM , allows calculation of the proportion of stone growth per total fruit growth:

$$\frac{d\text{DM}_s}{d\text{DM}} = \text{share}_1 \times \text{share}_2 \times e^{-\text{share}_2 \times \text{DM}} \quad (2)$$

where share_1 and share_2 are parameters. In this way, dry flesh and stone masses are calculated daily.

Sugar sub-model

The sugar sub-model was developed by Génard et al. (2003) from the model described by Génard and Souty (1996). It has been tested in different conditions (year, assimilate and water supply). It simulates carbon partitioning in peach flesh, during fruit growth, into several compounds: four sugars (sucrose, sorbitol, glucose and fructose) and other compounds in the fruit (e.g., starch and structural carbohydrates) and respired CO_2 . The incoming carbon flow is calculated by the carbon sub-model. The rates of change per unit time of amounts of carbon in the four sugar compounds depend on the sugars already accumulated in the fruit flesh. They are described through a set of differential equations, each equation being of the form:

$$\frac{dC_j}{dt} = E_j + \sum_{i \neq j} k_{ij}(\theta, x) C_i - C_j \sum_{i \neq j} k_{ij}(\theta, x) - R_j \quad (3)$$

where C_j is the amount of carbon in compartments j , E_j and R_j , which can be equal to zero depending on the compartment, representing the carbon flows from the phloem and the carbon loss by respiration, respectively; k_{ij} is a function of parameters (θ); and variables (x) describing the relative rate of transformation of sugar i into sugar j . According to the equations given by Génard et al. (2003), the relative rate of sucrose transformation into glucose and fructose depends on phenological time (day after full bloom) through an exponential equation; the relative rates of sorbitol transformation into fructose or glucose are constants; and the relative rate of transformation of either glucose or fructose into compounds other than sugars is modeled as being linearly related to the relative growth rate of flesh dry mass.

Water sub-model

The water sub-model is an adaptation of the biophysical model of fruit growth developed by Fishman and Génard (1998) and revised by Lescourret et al. (2001). The time course of water mass in the fruit flesh is calculated assuming that water enters the fruit flesh from xylem and phloem and is lost through transpiration. The flow of solution from xylem or phloem to the fruit cells follows a general law:

$$U = AL(P - P_f - \sigma(p - p_f)) \quad (4)$$

where “f” refers to fruit variables, A is the external surface area of the vascular network assumed to be proportional to fruit area A_f ($A = aA_f$, where a is a non-dimensional coefficient of proportionality), L is the hydraulic conductivity coefficient of vascular network membranes, P and p are the hydrostatic and osmotic pressures, respectively, and σ is a measure of impermeability of the membrane to solutes. In the case of xylem, water must cross the plasma membrane of cells, for which σ is close to 1 (Nobel 1974, Murphy and Smith 1994). Water can enter fruit cells from phloem through plasmodesmata (Patrick and Offler 1996). However, most plasmodesmata are closed, after the early stage of starch accumulation, in fleshy fruits such as grape (*Vitis vinifera* L.), peach or tomato (*Lycopersicon esculentum* Mill.), which accumulate soluble sugars to relatively high concentrations (Patrick and Offler 1996, Patrick 1997). Thus, σ was assumed to be close to one for both xylem and phloem.

The fruit osmotic pressure induced by sugars is calculated by means of the sugar sub-model outputs. The hydrostatic pressure of the fruit flesh is calculated by solving the Lockhart equation describing the growth in volume, V , of the fruit flesh (Lockhart 1965) as a function of pressure and current fresh mass:

$$\frac{dV}{dt} = \begin{cases} V\phi(P_f - Y), & \text{if } P_f > Y \\ 0, & \text{if } P_f \leq Y \end{cases} \quad (5)$$

where ϕ is extensibility of the cell walls and Y is the yield threshold value that the hydrostatic pressure of the fruit flesh has to exceed before irreversible expansion occurs. Assuming that the change in fruit flesh volume mainly results from water balance, it can also be calculated from Equation 6 as:

$$\frac{dV}{dt} = \frac{U_x + U_p - T_f}{D_w} \quad (6)$$

where “x” and “p” refer to xylem and phloem, D_w is water density and T_f is fruit transpiration rate. Under the condition of steady irreversible growth, Equations 5 and 6 must be equal. Setting them equal, and inserting the flux from Equation 4, the resulting equations for P_f can be solved. If the environmental conditions lead to low P_f values, the cell wall stresses are relieved and P_f is assumed to stay close to zero (Fishman and Génard 1998). In this case, Equation 5 is no longer valid and the change in fruit flesh volume is calculated from Equation 6. This change may be negative, so that the model takes into account that water can be exported from the fruit into other plant organs as shown by Greenspan et al. (1996).

The initial model ran on an hourly basis, considering the hourly variations in stem water potential, temperature and air humidity, the last two variables being implicated in fruit transpiration. When the virtual peach fruit model is run on a daily basis, hourly variation is not considered. For various water conditions, we compared model outputs using series of either hourly values of the three input variables, or daily values each corresponding to hourly (24) values averaged per day. With a slight variation in parameter a (Equation 4) (see Materials and methods), we obtained similar fresh mass curves (mean absolute difference about 2–4%). Averaged over a day, the osmotic pressures and the hydrostatic pressures greater than Y (Equation 5) were also similar (data not shown). Thus, the mean daily values of stem water potential, temperature and air humidity proved adequate for predicting the daily fruit water balance.

Determination of stone fresh mass, M_s , was required to compute the part of total mass consisting of flesh. It used stone dry mass calculated by the carbon sub-model, DM_s , and the following empirical relationship:

$$M_s = \text{stone}_1 \times DM_s \times \text{stone}_2 \quad (7)$$

where stone_1 and stone_2 are parameters.

The quality traits we considered were derived from the intermediate outputs of the combined model (flesh and stone dry masses, amounts of sugars and water mass in the fruit flesh, stone fresh mass; Figure 1). A sweetness index (g of equivalent sucrose per 100 g of flesh fresh mass) was computed as a linear combination of sugar concentrations, with the sweetness ratings of each sugar (Kulp et al. 1991) as coefficients.

Materials and methods

Calibrating and testing the virtual peach fruit model

Three experiments were performed in 1993, 1996 and 1997 on peach trees of the late maturing cv. ‘Suncrest/GF 677’ planted in 1982 at the INRA Centre of Avignon, France (43.9° N, 4.8° E). Trees were goblet-trained and received routine horticultural care, including non-limiting irrigation. Three leaf:fruit ratios (6, 18, 30 leaves per fruit) in 1993 and 1996, and two leaf:fruit ratios (10, 30 leaves per fruit) in 1997 were applied to 240 (1993, 1996) or 120 (1997) fruit-bearing stems

isolated from the rest of the tree by girdling, on 36–45 trees between mid-May and early June, depending on the year. Vegetative growth was prevented by removing the shoot tip. For every year and treatment, five replicates (made up of fruit from one to two similar shoots) were harvested weekly until fruit maturation. Each measure comprised fruit fresh mass, the fresh and dry masses of the stone, the dry matter content of flesh (evaluated after drying at 70 °C for 72 h), and the concentrations of the four sugars (% of flesh fresh mass). The sugars were measured by high performance liquid chromatography (HPLC) (see Génard and Souty 1996 and Génard et al. 2003). The measurements obtained for a replicate were averaged. The corresponding data, which have been published by Génard et al. (2003), were used to: (1) estimate parameter a implicated in water inputs (Equation 4) and parameters needed to compute the sharing of carbon between the fruit flesh and the stone and stone fresh mass (Equations 2 and 7); (2) test the goodness-of-fit of the virtual peach fruit model; and (3) evaluate the predictive quality of the peach fruit model.

During 1996, leafy shoots and 1-year-old stems were harvested at dawn (five replicates per treatment and harvest date), and analyzed to evaluate the amount of carbon reserves per unit mass. Soluble sugars and starch were determined as described by Gomez et al. (2002) and Jordan and Habib (1996), respectively. The carbon reserve data were used to estimate parameters of reserve mobilization in the carbon sub-model (see below).

Data from various experiments conducted from 1991 to 1997 on cv. ‘Suncrest’ at the INRA Centres of Avignon or Gotheron (120 km north of Avignon) were combined to create a database of regular measurements of fruit growth in dry mass (861 masses and 127 dates) in non-limiting carbon conditions (30 leaves per fruit or more). From this database, parameters of potential fruit growth in dry mass were estimated (see below). Data obtained in 1991 also comprised stone dry and fresh masses; they were added to data of the same type obtained in 1993, 1996 and 1997 to estimate the parameters of Equations 2 and 7.

Inputs of the virtual peach fruit model

Global radiation, temperature and relative humidity data (Figure 1) were collected by INRA weather stations close to the experimental fields. Because the experimental trees were well-watered, a daily value of -0.45 MPa was taken for stem water potential based on the mean of hourly default values for well-watered peach trees used by Fishman and Génard (1998), from which the water sub-model originates. Between- and within-shoot shadow coefficients to compute photosynthesis (Figure 1) were mean values extracted from databases constituted for cv. ‘Suncrest’ on the basis of fisheye photographs (see carbon sub-model description). The 1996 data were used to initialize the stem and shoot variables for every simulation. For simulations corresponding to given experimental conditions (year, leaf:fruit ratio), the fruit variables were initialized by averaging the data observed in these conditions on the first date of measurement.

Parameter estimation

The original values of most of the dparameters of the sub-models were taken from Fishman and Génard (1998), Lescourret et al. (1998, 2001) and Génard et al. (2003), with the following exceptions.

Parameters of reserve mobilization in the carbon sub-model, r_4 (leafy shoot mobile fraction of reserves) and r_5 (1-year-old stem mobile fraction of reserves) were taken from the literature in the original carbon sub-model (Lescourret et al. 1998). Here they were estimated with the 1996 reserve data, by minimizing a weighted sum of the MSE (mean squared error, i.e., mean squared difference between observed and model values) for each of the six combinations of reserve compartment (stem or leaf) and leaf:fruit ratio (6, 18 or 30). The MSE for each combination was weighted by the inverse of the variance of the corresponding data. The estimated values were $r_4 = 2.6 \times 10^{-2} \text{ day}^{-1}$ and $r_5 = 5 \times 10^{-4} \text{ day}^{-1}$ ($n = 56$).

A preliminary estimation of parameters of potential fruit growth in dry mass, dd_{\min} , dd_{\max} , RGR^{ini} and DM^{max} (Equation 1) was presented in Lescourret et al. (1998). However, initial tests of the virtual peach fruit model showed that it was necessary to revise these estimates. The dd_{\max} was estimated as the maximal value observed in the previously mentioned database (1800 dd). The other parameters were estimated by nonlinear least squares regression, using 90 masses for 31 dates corresponding to the higher values in the data set (90% quantile at each date). Estimated values were $dd_{\min} = 839 \text{ dd}$ (SE = 270 dd), $RGR^{\text{ini}} = 4.04 \times 10^{-3} \text{ dd}^{-1}$ (SE = $1.64 \times 10^{-4} \text{ dd}^{-1}$) and $DM^{\text{max}} = 59.22 \text{ g}$ (SE = 10.71 g, $n = 90$).

Parameters $share_1$ and $share_2$ (Equation 2), and $stone_1$ and $stone_2$ (Equation 7) were specific to the combined model. They were estimated by nonlinear or linear regressions. Estimated values were $share_1 = 5.80 \text{ g}$ (SE = 0.089 g), $share_2 = 0.10 \text{ g}^{-1}$ (SE = 0.004 g^{-1} , $n = 677$), $stone_1 = 1.17$ (dimensionless, SE = 0.035) and $stone_2 = 3.82 \text{ g}$ (SE = 0.15 g, $n = 677$).

Parameter a in the water sub-model (Equation 4) had been subjected to a calibration procedure in the original biophysical model of fruit growth (Fishman and Génard 1998). Therefore, we reestimated it by minimizing the sum of the MSE over the eight available data sets (combinations of year and leaf:fruit ratio, with 3 years and two to three leaf:fruit ratios). The target of this calibration was the dry matter content of the flesh. The estimated value was 2.66×10^{-2} (dimensionless, SE = 5.7×10^{-4} , $n = 391$).

Goodness-of-fit and predictive quality of the virtual peach fruit model

To test the goodness of fit of the virtual peach fruit model, the relative root mean squared error (RRMSE) (Kobayashi and Us Salam 2000) was calculated separately for each of the eight combinations of year \times leaf:fruit ratio and each of the quality traits as:

$$\frac{1}{\bar{y}} \sqrt{\frac{\sum_{i=1}^N n_i (y_i^c - \bar{y}_i)^2}{\sum_{i=1}^N n_i}} \quad (8)$$

where N is the number of dates over the growing period, n_i is the number of fruit-bearing stems measured at date i , y_i^c is the value of the quality trait at date i calculated by the model, \bar{y}_i is the average of values for the “mean” fruits corresponding to n_i shoots, and \bar{y} is the mean of all observed values.

The predictive quality of the virtual peach fruit model, which evaluates the validity of the model outside its range of development, was computed for each fruit quality trait. The criterion was a relative root mean squared error of prediction (RRMSEP). The classical MSEP (mean squared error of prediction) was first computed based on the eight experimental data sets (year \times leaf:fruit ratio combinations), by cross-validation (cf. Batchelor et al. 1994, Wallach et al. 2001). Cross-validation is a common approach to estimating prediction error. It is a more objective alternative to splitting the data, using part for adjustment and part for evaluation, because the way data are split influences both estimation and prediction error (Wallach et al. 2001). The principles of cross-validation are as follows (Wallach et al. 2001). Two data sets are made: one set contains a single situation (the target situation, in our case a single combination of year and leaf:fruit ratio), and the other, all the data independent of the target situation (in our case, not from the same year). Parameters are fitted using the second data set. In our case, just five parameters were fitted in this way because their estimation was largely based on the eight situations: $share_1$, $share_2$, $stone_1$, $stone_2$ and a (Equations 2, 4 and 7). The error of the resulting model is calculated based on the target situation. The procedure is repeated using every situation in turn as the target situation, and averaging the errors over all the target situations gives an estimate of the prediction error.

Design of theoretical experiments

In the first theoretical experiment, three factors were varied together: weather, leaf:fruit ratio and stem water potential. For weather, 11 data series extracted from the INRA climatic database (years 1992 to 2002 at Avignon) were analyzed by principal component analysis followed by hierarchical clustering on the main components, yielding two main classes. Two contrasting climatic years representative of these classes (1997 and 1998) were chosen as levels for the “weather” factor. Compared with 1997, 1998 was on average (based on daily values) $2 \text{ }^\circ\text{C}$ warmer, received about $200 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ more global radiation (daily averaged hourly photosynthetic photon flux), and was less humid (mean relative humidity of 0.52 compared with 0.68 for 1998). For leaf:fruit ratio, the three values chosen were 6, 18 and 30 leaves per fruit. Stem water potential daily values (see water sub-model description) corresponded to normal watering (-0.45 MPa) or water stress. In the latter case, different values were attributed to the three leaf:fruit ratios (-0.65 , -0.79 and -0.92 MPa for 30, 18 and 6 leaves per fruit, respectively) because stem water potential varies according to crop load (Berman and DeJong 1996). These values were calculated by averaging hourly values proposed by Fishman and Génard (1998) for these water and crop load conditions. The simulations (one per combination of factor levels) took place from 81 to 139 days after bloom, each factor level was kept constant during the simulation period. The sensitivity of the virtual peach fruit model to factors was

assessed by contributions to the sum-of-squares resulting from variance analysis, considering the main effects of the three factors plus time as a linear numerical covariate.

In the second theoretical experiment, four time-scenarios of conditions of stem water potential were studied. In two scenarios, the water conditions were kept constant throughout the simulation period (81–139 days after bloom). They corresponded to conditions of normal watering or water deficit (–0.45 and –0.79 MPa as daily values for stem water potential, respectively). The two other scenarios, which used the same values of stem water potential, were a period of normal watering followed by a period of water deficit of the same duration, and conversely a period of water deficit followed by a period of normal watering of the same duration. Weather was that of year 1997, and leaf:fruit ratio was 18 leaves per fruit.

In the third theoretical experiment, four time-scenarios of leaf:fruit ratio were studied. In two scenarios, the leaf:fruit ratio was kept constant throughout the simulation period (81–139 days after bloom): 30 and 6 leaves per fruit, respectively. In two other scenarios, the simulation period was split into two equal parts, with either 30, then 6 leaves per fruit (which may be obtained by leaf removal), or 6, then 30 leaves per fruit (which may be obtained by fruit removal). Weather was that of year 1997 and daily stem water potential was –0.45 MPa.

The three experimental designs were not intended to represent real situations—keeping water stress constant or setting up time-scenarios of water or leaf:fruit ratio such as those described here would be difficult if not impossible. The three experimental designs were intended solely as a theoretical framework to explore the behavior of a modeled system.

All statistical analyses were performed with the S-plus statistical package (Version 3.4, release 1 for Sun SPARC, SunOS 5.3: 1996).

Results and discussion

Goodness-of-fit and predictive quality of the virtual peach fruit model

The virtual peach fruit model simulated the order of magnitude and seasonal variations of reserve content for three leaf:fruit ratios (Figure 2) and of quality traits for several leaf:fruit ratios and the 3 years (Figure 3). According to the goodness-of-fit criteria (Table 1), there was good agreement between model and data for the proportion of flesh in the total mass. The agreement was also quite good for fruit fresh mass and dry matter content of the flesh. Larger model errors were found for flesh concentrations of sugars and the sweetness index. The largest relative errors were for sorbitol, and were partly associated with the low concentrations of this compound.

The predictive quality of the virtual peach fruit model was good: the RRMSEP values, which ranged from 0.03 to 0.44 (in the case of sorbitol concentration), were close to the mean of RRMSE values and followed the same hierarchy (Table 1).

To what extent is the virtual peach fruit model sensitive to environmental factors?

The first theoretical experiment, which involved the factors

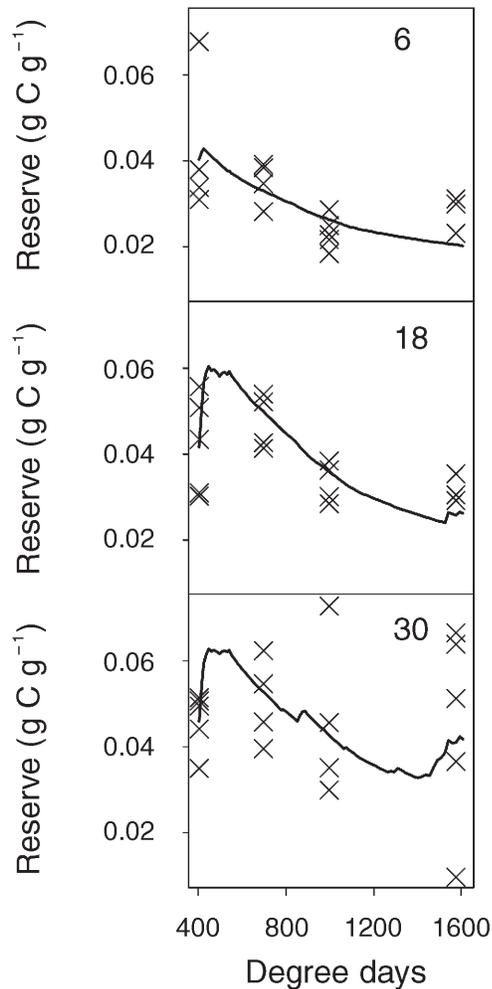


Figure 2. Model simulations (lines) versus data (crosses) depicting total fruit-bearing stem reserves. Data were collected in 1996 for leaf:fruit ratios of 6, 18 or 30 leaves per fruit.

weather, leaf:fruit ratio and stem water potential, with time as a covariate, was intended to answer this question. Here we restrict examination of the virtual peach fruit model response to some important physiological and quality variables. The physiological variables were: the photosynthesis rate per unit leaf area that depicts carbon source activity; the fruit carbon demand per fruit that depicts carbon sink activity; the fruit hydrostatic pressure that determines fruit growth in fresh mass according to Lockhart equation implemented in the water sub-model; and the relative rate of synthesis of compounds other than sugars from fructose and glucose that is indicative of the intensity of sugar metabolism in the fruit. The quality traits were fruit fresh mass, the proportion of flesh in the total mass, the dry matter content of the flesh, and the sweetness index.

The range of quality traits over the treatments was wide: at the end of the simulation, values ranged from 67 to 182 g for fresh mass, 0.86 to 0.94 for the proportion of flesh, 0.11 to 0.18 for dry matter content of the flesh, and 5.5 to 10% for the sweetness index. The factor contributions to the total sum-of-squares following analysis of variance are presented in Ta-

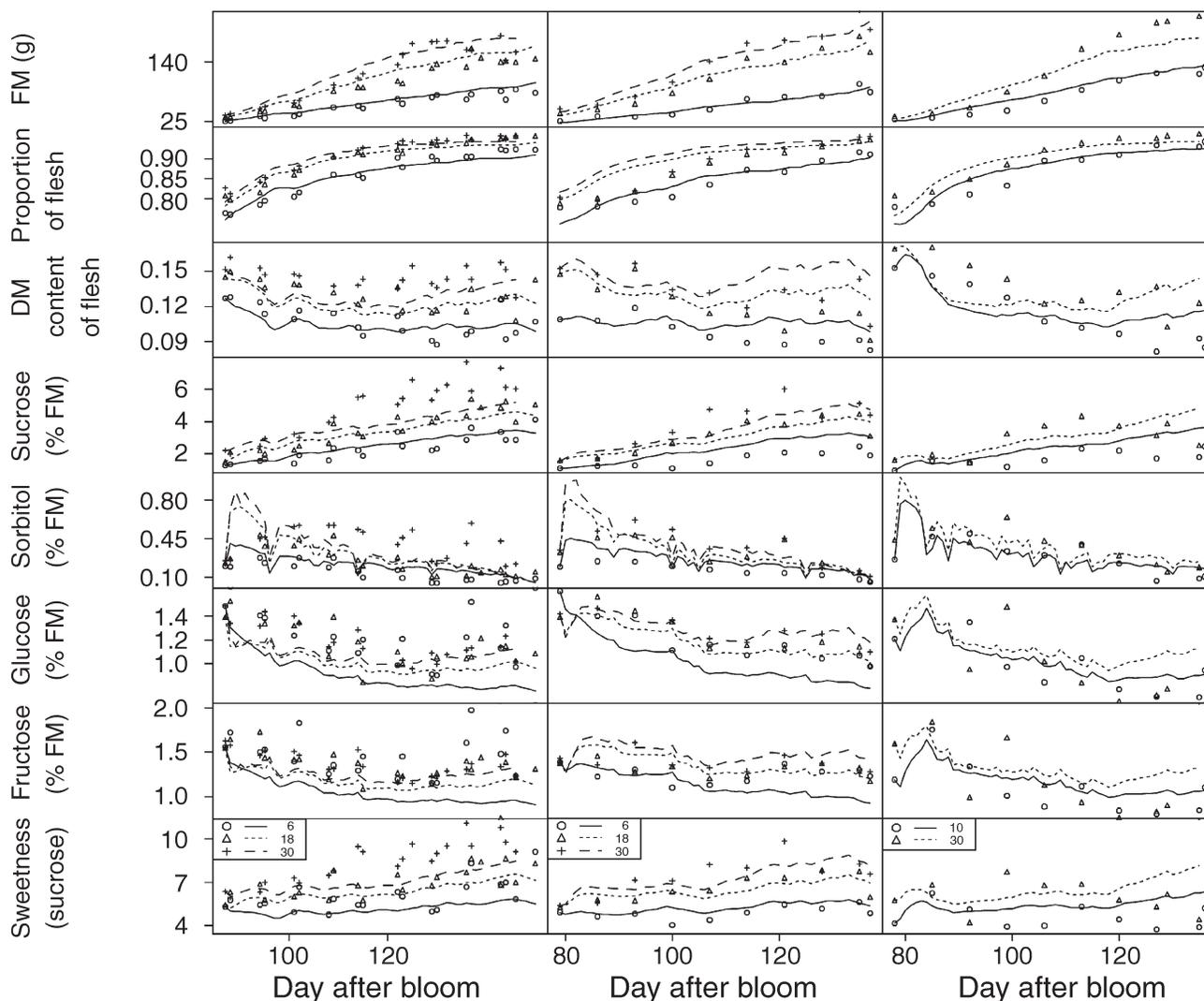


Figure 3. Time courses of mean observed (points) and simulated (lines) quality traits for 3 years (from left to right: 1993, 1996, 1997) and different leaf:fruit ratios (6, 10, 18, 30). The observed values are from Génard et al. (2003). Abbreviation: FM = fresh mass.

ble 2. Some striking points emerge from the results. First, all the variables studied but two (photosynthetic rate and dry matter content of the flesh) are developmentally regulated, so that time (i.e., days after bloom) made a large contribution to their

Table 1. Goodness-of-fit (RRMSE) and predictive quality (RRMSEP) of the virtual peach fruit model. Abbreviation: DM = dry matter.

| Quality trait | Mean and range of RRMSE | RRMSEP |
|---------------------------------------|-----------------------------------|--------|
| Fresh mass | 0.14 (0.10–0.20) | 0.16 |
| Proportion of flesh in the total mass | 0.016 (2×10^{-4} –0.09) | 0.03 |
| DM content of flesh | 0.13 (0.09–0.16) | 0.13 |
| Sucrose concentration | 0.29 (0.15–0.54) | 0.29 |
| Sorbitol concentration | 0.42 (0.28–0.60) | 0.44 |
| Glucose concentration | 0.18 (0.07–0.33) | 0.20 |
| Fructose concentration | 0.18 (0.08–0.33) | 0.22 |
| Sweetness | 0.18 (0.08–0.28) | 0.20 |

variation. Second, the virtual peach fruit model is clearly sensitive to environmental factors, which accounted together for 26–80% and 8–65% of the total sum-of-squares for quality traits and physiological variables, respectively. However, the effect of weather was unimportant compared with other factors, which is in agreement with the small year effect found in experimental studies on apple (*Malus domestica* Borkh.) and peach fruit quality (Robinson et al. 1991, Génard et al. 2003). Leaf:fruit ratio contributed highly to photosynthetic rate and to a lesser extent to fruit demand, and consequently to fruit metabolism, including osmotic potential that was lower for high ratios (data not shown). As a consequence, the mass of water in the fruit, and thus the fresh mass, increased with leaf:fruit ratio, as in the experiment of Berman and DeJong (1996) and the observations of McFadyen et al. (1996). The effects of leaf:fruit ratio on both dry matter content of the flesh and sweetness index were marked for well-irrigated fruits, in accordance with previous experiments (Génard et al. 2003), but almost non-existent in the case of water-stressed plants (data not

Table 2. Contribution of time (day after bloom, DAB) and factors (percent of sum-of-squares following variance analysis) to some variables of the virtual peach fruit model in a theoretical experiment. The first five columns are physiological variables, the last four columns are quality traits. Abbreviations: df = degrees of freedom; PR = Photosynthetic rate; EFD = early fruit demand; LFD = late fruit demand; P = hydrostatic pressure; FM = fresh mass; DM = dry matter; and Ψ_{stem} = stem water potential.

| Explanatory variable (df) | PR | EFD ¹ | LFD ¹ | Fruit P > 0.5 MPa ² | Synthesis (other than sugar) | Fruit FM | Proportion of the flesh | DM content of the flesh | Sweetness index |
|---------------------------|-------|------------------|------------------|----------------------------------|------------------------------|----------|-------------------------|-------------------------|-----------------|
| Day after bloom (1) | 8.10 | 31.62 | 21.82 | 38.04 | 52.34 | 62.49 | 62.48 | 0.42 | 28.25 |
| Leaf:fruit ratio (2) | 62.02 | 11.08 | 33.39 | 6.14 | 23.83 | 20.74 | 15.34 | 0.2 | 4.47 |
| Ψ_{stem} (1) | 0 | 0 | 0 | 1.68 | 0 | 5.90 | 10.32 | 68.82 | 45.26 |
| Weather (1) | 2.82 | 6.46 | 0.09 | 0.05 | 0.09 | 0.37 | 0.40 | 10.02 | 8.71 |
| Residuals (690) | 27.06 | 50.84 | 44.7 | 54.09 | 23.74 | 10.5 | 11.46 | 20.54 | 13.31 |

¹ For fruit demand, two variance analyses were undertaken because of the bell-shaped response of this variable to DAB: for EFD, DAB \leq 110; and for LFD, DAB > 110.

² Only pressures > 0.5 MPa, the threshold value above which fruit growth occurs according to the water sub-model of the virtual peach fruit model (Fishman and Génard 1998), were considered.

shown). Accordingly, the contribution of leaf:fruit ratio alone was weak, whereas the contribution of its interaction with stem water potential was greater (11 and 6% for dry matter content and sweetness, respectively). Stem water potential contributed substantially to the dry matter content of the flesh and to the sweetness index. This result is consistent with many experimental findings showing increases in fruit soluble solids or sugar concentrations with water stress (Crisosto et al. 1994, Ginestar and Castel 1996, Yakushiji et al. 1998, Chartzoulakis et al. 1999). Third, the contribution of factors was much less for the physiological variables than for the quality traits. In contrast with quality traits, which are at every time-step the result of a history (e.g., the fruit fresh mass is a cumulated value), the physiological variables are either highly regulated

(e.g., hydrostatic pressure) or instantaneous and sensitive to day-to-day variation within a weather series, which did not contribute to the variance analysis.

Can the virtual peach fruit model generate simple, general laws?

To answer this question, we use the results of the first theoretical experiment, where various model inputs varied, to examine relationships between quality traits, between physiological variables, and between both. Considering all the data from the first theoretical experiment, a strong link was found between fruit fresh mass and the proportion of flesh in the total mass, and there was a weaker link between dry matter content of the flesh and the sweetness index (Figure 4). Fruit fresh mass and

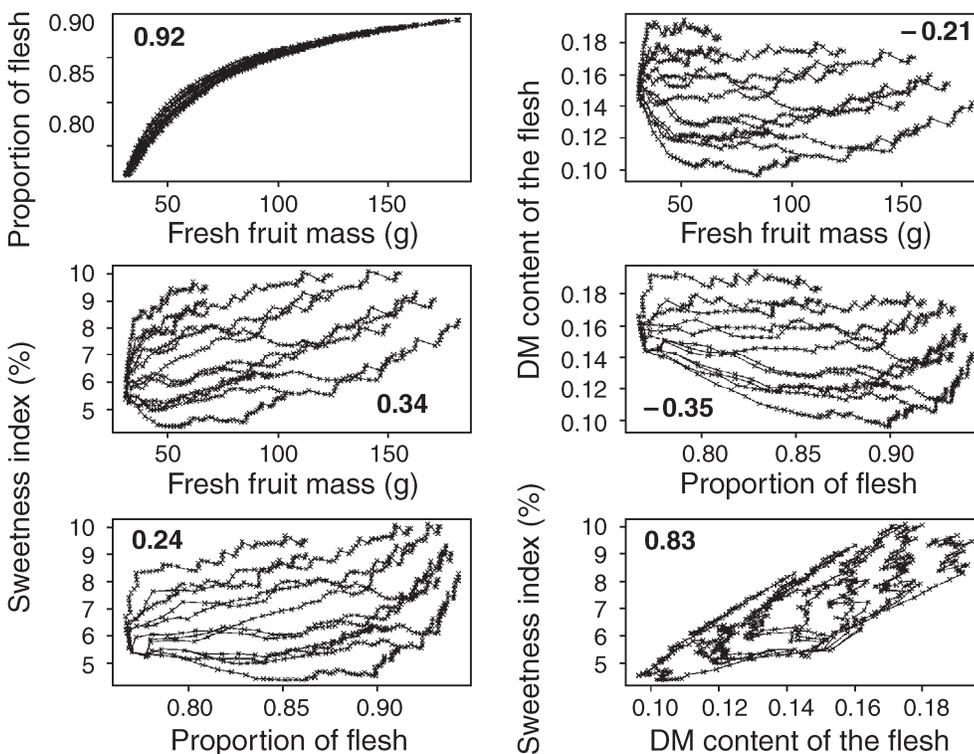


Figure 4. Pairwise plots of quality traits in the first experiment performed with the virtual peach fruit model. Each plot gathers data of the 12 combinations of factor levels (three leaf:fruit ratios, two weathers and two stem water potentials). Lines relate data (crosses) simulated each day (simulation period = 81–139 days after bloom) for a given combination of factor levels. Pearson correlations are indicated.

the proportion of flesh were also closely linked within every factor combination leaf:fruit ratio \times stem water potential \times weather (Pearson correlation = 0.95 ± 0.02), partly because both variables were strongly time-dependent (Table 2). Similarly, a unique relationship between flesh and fruit masses was obtained from experimental data by Léchaudel et al. (2002) on mango fruit (*Mangifera indica* L. cv. 'Lirfa'), for five leaf:fruit ratio treatments ranging from 10 to 150 leaves per fruit. The second link was approximately linear, with fairly large variation. However, correlations between dry matter content of the flesh and sweetness varied from -0.05 to 0.88 among the factor combinations. The most notable feature of this link is that low dry matter content is never associated with high sweetness index, or high dry matter content with low sweetness index. For the other pairs of quality traits, though correlations could be strong within factor combinations, no clear overall pattern emerged from the data.

Among physiological variables (Figure 5), only two relationships were sufficiently strong to merit mention. A positive link was found between fruit carbon demand per unit leaf area and photosynthetic rate, with a strong increase in photosynthetic rate with fruit carbon demand for demand values less than 10 g C m^{-2} , then a slower increase with a plateau at about 5.5 g C m^{-2} . This relationship was time independent. It is valid for the conditions of the simulation, i.e., the case where only local assimilates, those of the fruit-bearing stem, are considered (see Materials and methods). A similar curve was observed by Ben Mimoun et al. (1996) in the case of experimental data obtained from fruit-bearing stems isolated from the tree by girdling, corresponding to two peach genotypes, 3 years and 5 to 50 leaves per fruit. The second positive link was a linear relationship between carbon demand per fruit and relative rate of synthesis of compounds other than sugars, the slope of which decreased with days after bloom. A likely explanation for this pattern is that periods of intense fruit demand are marked by the synthesis of new structures such as cell walls, whereas synthesis of new structures diminishes as the fruit approaches maturity (Bouranis and Nivis 1992, Fishman et al. 1993).

Physiological variables, which are instantaneous, were not related directly to quality traits, but to their rate of change per unit time. Significant relationships were found with fruit hydrostatic pressure only (Figure 6). Rates of change of both fruit fresh mass and the proportion of flesh increased with fruit hydrostatic pressure, whereas those of both dry matter content of the flesh and sweetness decreased. According to the Lockhart law implemented in the model, small increments in hydrostatic pressure are sufficient to increase the rate of change of flesh mass, so that ratios per unit flesh mass, like dry matter content or sweetness, decrease.

Overall, the virtual peach fruit model suggests that simple and general laws are common. In this investigation, pairwise scatter plots of virtual peach fruit model variables demonstrated relationships in about one third of cases.

Can the virtual peach fruit model produce complex behavior?

The second theoretical experiment compared four time-scenarios of stem water potential, denoted here as W (constant

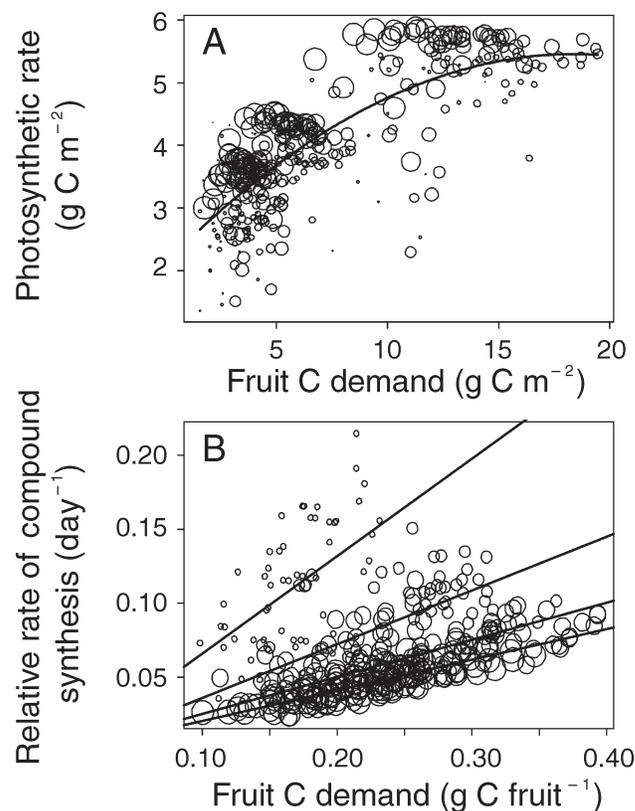


Figure 5. Relationships between fruit carbon demand and either photosynthetic rate (A, with a smooth curve), or relative rate of synthesis of compounds other than sugars (B, with regression lines for four classes of days after bloom: ≤ 90 , 90–100, 100–120, > 120). Symbols are proportional to days after bloom. Data are from the first experiment performed with the virtual peach fruit model, with leaf:fruit ratio, weather and stem water potential as factors.

normal water conditions), W/S (normal water conditions, then water stress, for two equal periods), S/W (water stress, then normal water conditions, for two equal periods) and S (constant water stress). These scenarios produced a wide range of responses of quality traits (Figure 7). The response to the S/W scenario looked like “compensatory growth” after re-watering, because growth loss during the stress period was fully regained after re-watering. A decrease in sweetness and dry matter content and an increase in the proportion of flesh were consequences of this compensatory growth. According to Trevas (2003), compensatory growth is a corrective mechanism involving feedback control to achieve a developmental goal. We hypothesized that the virtual peach fruit model mimics such a mechanism based on a “sugar signal,” assuming that the increase in sugar concentration during the stress period (as shown in Figure 7 for the sweetness index) promotes growth after re-watering.

Application of water stress after a period of normal water conditions (W/S) resulted in a sharp slowdown in growth. During the same period, fruit that encountered continuous water stress (S) experienced continuous growth, implying that the S fruit had become adapted to drought compared with the W/S fruit. In real plants, this kind of adaptation has been called a

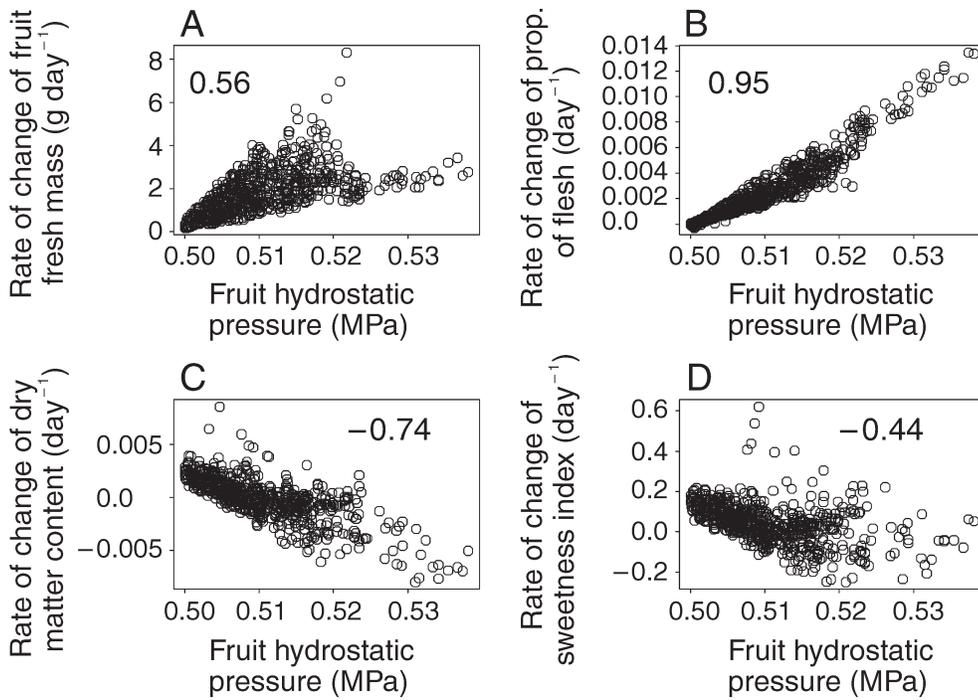


Figure 6. Relationships between fruit hydrostatic pressure (≥ 0.5 MPa) and rates of change per unit time of fruit fresh mass (A), proportion of flesh in the total mass (B), dry matter content of the flesh (C) and sweetness index (D). Data are from the first experiment performed with the virtual peach fruit model, with leaf:fruit ratio, weather and stem water potential as factors. Pearson correlations are indicated.

memory effect (Trewavas 2004). We can relate growth patterns to sugar concentration patterns. Sugar concentration was much higher in S plants than in W/S plants at 110 days after bloom, the beginning of the stress period for W/S. We therefore hypothesized that the high sugar concentration allowed fruit growth under conditions of water deficit.

The third theoretical experiment compared four time-scenarios of leaf:fruit ratios, denoted here as 30 (30 leaves per fruit during the entire simulation period), 30/6 (30, then 6 leaves per fruit, for two equal periods), 6/30 (6, then 30 leaves per fruit, for two equal periods) and 6 (6 leaves per fruit during the

entire simulation period). The treatments resulted in contrasting responses of quality traits (Figure 8). During a period of 10 days after changing leaf:fruit ratio, i.e., from 110 to 120 days after bloom, sugar concentration and dry matter content changed markedly, decreasing or increasing in the same direction as leaf:fruit ratio, as shown on Figure 8. In the 30/6 and 6/30 scenarios, the responses of fresh mass and of the proportion of flesh to changing leaf:fruit ratio exhibited time lags of 5–10 days. A possible explanation is that, for the 30/6 scenario, the sugar concentration was high enough to maintain rapid growth for a few days after changing the leaf:fruit ra-

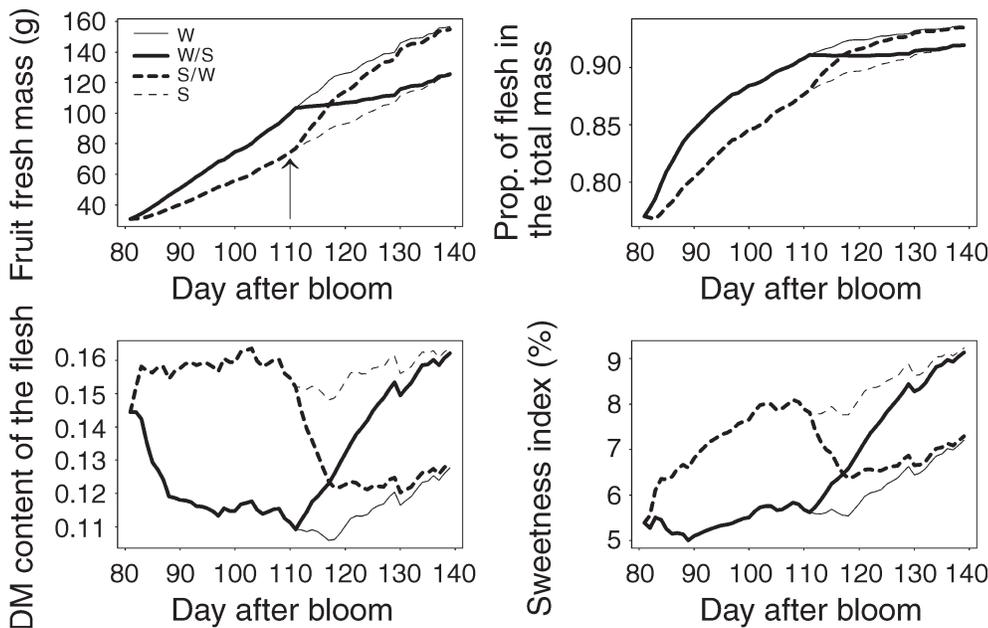


Figure 7. Time courses of four quality traits as predicted by the virtual peach fruit model, under four time-scenarios of water conditions (W = normal water conditions, W/S = normal water conditions, then water stress, S/W = water stress, then normal water conditions, and S = water stress). The arrow indicates the time when water conditions change in the W/S and S/W scenarios. Abbreviations: DM = dry matter; and Prop. = proportion.

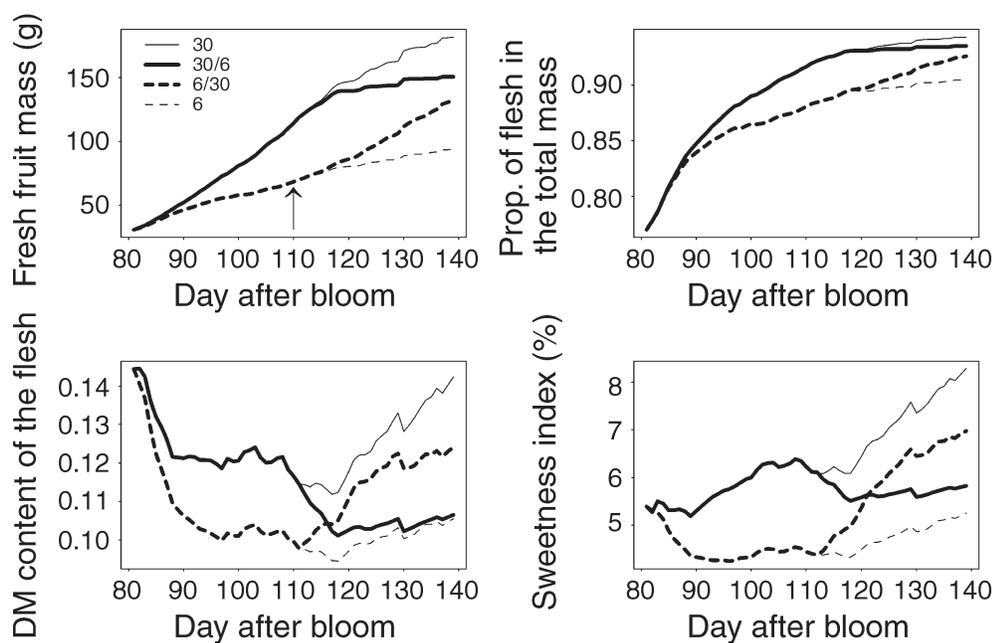


Figure 8. Time courses of four quality traits as predicted by the virtual peach fruit model, under four time scenarios of leaf:fruit ratio (30 = 30 leaves per fruit, 30/6 = 30 then 6 leaves per fruit, 6/30 = 6 then 30 leaves per fruit, 6 = 6 leaves per fruit). The arrow indicates the time when leaf:fruit ratio changes in the 30/6 and 6/30 scenarios.

ratio, whereas in the 6/30 scenario, the sugar concentration was insufficient to immediately increase fruit growth rate. By 120 days after bloom, the responses of every quality trait to the 30/6 and 6 treatments on the one hand, to the 6/30 and 30 treatments on the other hand, were parallel, suggesting that the rate of change of every quality trait depended only on the current leaf:fruit ratio.

In the second theoretical experiment, the W and S/W treatments and the S and W/S treatments produced similar final results for every quality trait (Figure 7). This suggests that, when simulated fruit growth undergoes several periods with different water conditions, the conditions of the last period are the most important. In the third theoretical experiment, all leaf:fruit scenarios produced different results (Figure 8), suggesting that the entire time course of assimilate supply was important in this particular case.

Conclusions

To our knowledge, the virtual peach fruit model is the first model to integrate in a systemic framework knowledge of many interrelated processes, resulting in a complex quality profile and emergent properties that are typical of complex systems. The adaptation to water stress, the compensatory growth, and the inertia to changing carbon supply were behaviors that were not predicted at the model design stage. The model offers various possibilities as a research tool, both for performing theoretical experiments and for helping to understand experimental results when studying the effects of technical scenarios for which there is no literature. We examined relationships between quality traits, between physiological variables and between both and found patterns that did not derive just from the aggregation of modeled basic functions. Moreover, the virtual peach fruit model may help in assessing the relative importance of processes for a given complex function or trait. For example, sugar metabolism is generally con-

sidered to be the main process leading to sugar concentration, but this assumption has never been verified. The virtual peach fruit model is a powerful tool for studying the respective contributions of sugar unloading, dilution by water and metabolism.

Virtual plants are being viewed as a novel means to simulate the genetic variability of plant responses to environmental conditions (Tardieu 2003). Combining either gene regulatory networks or quantitative trait loci (QTL) and models (Reymond et al. 2003) are two possible avenues, assuming that a genotype is represented by one parameter set (Tardieu 2003). Analyzing genotypic variation by means of models is a first step, and has been done with the carbon sub-model on a contrasting population of peach genotypes (Quilot et al. 2002). The results show that the main process explaining genotypic fruit growth variations is fruit growth demand, allowing for a restricted subsequent QTL analysis of carbon model parameters. This work is being completed and pursued using the virtual peach fruit model. In addition, incorporating the virtual peach fruit model in a crop model (work in progress) will be of value in simulating the combined effects of changes in climate, pest events changing leaf area or photosynthesis, genotype and technical operations on profiles of quality traits, and will thereby help to improve breeding and crop management processes.

Acknowledgments

We are indebted to R. Laurent and P. Rouet for field assistance, L. Gomez, E. Rubio and D. Dumont for starch and sugar analyses. Many thanks are owed to A. Hall for revising the manuscript, suggesting modifications and correcting the English. This work was partially funded by grants from Ministère de la Recherche, Programme Aliment-Qualité-Sécurité, décision d'aide No. 99 P0497; Conseil Régional Provence-Alpes-Côte d'Azur, arrêté No. 99/11831; INRA national program No. 67 Action Transversale Production Fruitière Intégrée.

References

- Abdel-Razik, M. 1989. A model of the productivity of olive trees under optimal water and nutrient supply in desert conditions. *Ecol. Model.* 45:179–204.
- Batchelor, W.D., J.W. Jones, K.J. Boote and G. Hoogenboom. 1994. Carbon-based models to predict peanut-pod detachment. *Trans. ASAE* 37:1639–1646.
- Baumgaertner, J., B. Graf and P. Zahner. 1984. A stochastic population model to simulate the annual growth pattern of mature Golden Delicious apple tree. *Rech. Agron. Suisse* 23:489–501.
- Ben Mimoun, M., J.J. Longuenesse and M. Génard. 1996. P_{max} as related to leaf:fruit ratio and fruit assimilate-demand in peach. *J. Hortic. Sci.* 71:767–775.
- Ben Mimoun, M., F. Lescourret, M. Génard and R. Habib. 1999. Modeling carbon allocation in peach shoot bearing fruits: simulation of the water stress effect. *Fruits* 54:129–134.
- Berman, M.E. and T.M. DeJong. 1996. Water stress and crop load effects on fruit fresh and dry weights in peach (*Prunus persica*). *Tree Physiol.* 16:859–864.
- Bouranis D.L. and C.A. Nivavis. 1992. Cell wall metabolism in growing and ripening stone fruits. *Plant Cell Physiol.* 33:999–1008.
- Bussièrès, P. 1993. Potential dry matter and water import rates in the tomato fruit in relationship to fruit size. *Ann. Bot.* 72:63–72.
- Bussièrès, P. 1994. Water import in tomato fruit: a resistance model. *Ann. Bot.* 73:75–82.
- Bussièrès, P. 1995. Dry matter and water import rates in the tomato fruit: a model incorporating the change in sap viscosity and osmotic potential with temperature. *Ann. Bot.* 77:469–476.
- Bussièrès, P. 2002. Water import in the young tomato fruit limited by pedicel resistance and calyx transpiration. *Funct. Plant Biol.* 29: 631–641.
- Buwalda, J.G. 1991. A mathematical model of carbon acquisition and utilization by kiwifruit vines. *Ecol. Model.* 57:43–64.
- Chartzoulakis, K., N. Michelakis and E. Stefanoudaki. 1999. Water use, growth, yield and fruit quality of 'Bonanza' oranges under different soil water regimes. *Adv. Hortic. Sci.* 13:6–11.
- Crisosto, C.H., R.S. Johnson, J.G. Luza and G.M. Crisosto. 1994. Irrigation regimes affect fruit soluble solids concentrations and rate of water loss of "O'Henry" peaches. *HortScience* 29:1169–1171.
- Fishman, S., B. Levaj, D. Gillespie and R. Scorza. 1993. Changes in the physicochemical properties of peach fruit pectin during on-tree ripening and storage. *J. Am. Soc. Hortic. Sci.* 118:343–349.
- Fishman, S. and M. Génard. 1998. A biophysical model of fruit growth: simulation of seasonal and diurnal dynamics of mass. *Plant Cell Environ.* 21:739–752.
- Génard, M. and J.G. Hugué. 1996. Modeling the response of peach fruit to water stress. *Tree Physiol.* 16:407–415.
- Génard, M. and F. Lescourret. 2004. Modeling fruit quality: ecophysiological, agronomical and ecological perspectives. *In* Production Practices and Quality Assessment of Food Crops. Vol. 1. Preharvest Practice. Eds. R. Dris and S.M. Jain. Kluwer Academic Press, Dordrecht, The Netherlands, pp 47–82.
- Génard, M. and M. Souty. 1996. Modeling the peach sugar contents in relation to fruit growth. *J. Am. Soc. Hortic. Sci.* 121:1122–1131.
- Génard, M., F. Lescourret, M. Ben Mimoun, J. Besset and C. Bussi. 1998. A simulation model of growth at the shoot bearing fruit level. II. Test and effect of source and sink factors in the case of peach. *Eur. J. Agron.* 9:189–202.
- Génard, M., F. Lescourret, L. Gomez and R. Habib. 2003. Changes in fruit sugar concentrations in response to assimilate supply, metabolism and dilution. A modeling approach applied to peach fruit (*Prunus persica* (L.) Batsch). *Tree Physiol.* 23:373–385.
- Ginestar, C. and J.R. Castel. 1996. Responses of young Clementine citrus trees to water stress during different phenological periods. *J. Hortic. Sci.* 71:551–559.
- Gomez, L., E. Rubio and M. Augé. 2002. A new procedure for extraction and measurement of soluble sugars in ligneous plants. *J. Sci. Food Agric.* 82:360–369.
- Greenspan, M.D., H.R. Schultz and M.A. Matthews. 1996. Field evaluation of water transport in grape berries during water deficits. *Physiol. Plant.* 97:55–62.
- Grossman, Y.L. and T.M. DeJong. 1994. Peach: a simulation model of reproductive and vegetative growth in peach trees. *Tree Physiol.* 14:329–345.
- Gutierrez, A.P., D.W. Williams and H. Kido. 1985. A model of grape growth and development: the mathematical structure and biological considerations. *Crop Sci.* 25:721–728.
- Heuvelink, E. and N. Bertin. 1994. Dry-matter partitioning in a tomato crop: comparison of two simulation models. *J. Hortic. Sci.* 69: 885–903.
- Jordan, M.O. and R. Habib. 1996. Mobilizable carbon reserves in young peach trees as evidenced by trunk girdling experiments. *J. Exp. Bot.* 47:79–87.
- Kobayashi, K. and M. Us Salam. 2000. Comparing simulated and measured values using mean squared deviation and its component. *Agron. J.* 92:345–352.
- Kulp, K., K. Lorenz and M. Stone. 1991. Functionality of carbohydrate ingredients in bakery products. *Food Technol.* 45:136–142.
- Léchaudel, M., M. Génard, F. Lescourret, L. Urban and M. Jannoyer. 2002. Leaf-to-fruit ratio affects water and dry matter content of mango fruit. *J. Hortic. Sci. Biotech.* 77:773–777.
- Lee, D.R. 1990. A unidirectional water flux model of fruit growth. *Can. J. Bot.* 68:1286–1290.
- Lescourret, F., M. Ben Mimoun and M. Génard. 1998. A simulation model of growth at the shoot bearing fruit level. I. Description and parameterization for peach. *Eur. J. Agron.* 9:173–188.
- Lescourret, F., M. Génard, R. Habib and S. Fishman. 2001. Variation in surface conductance to water vapor diffusion in peach fruit and its effects on fruit growth assessed by a simulation model. *Tree Physiol.* 21:735–741.
- Lobit, P., M. Génard, B.H. Wu, P. Soing and R. Habib. 2003. Modeling citrate metabolism in fruits: response to growth and temperature. *J. Exp. Bot.* 54:2489–2501.
- Lockhart, J.A. 1965. An analysis of irreversible plant cell elongation. *J. Theor. Biol.* 8:264–275.
- McFadyen, L.M., R.J. Hutton and E.W.R. Barlow. 1996. Effects of crop load on fruit water relations and fruit growth in peach. *J. Hortic. Sci.* 71:469–480.
- Murphy, R. and J.A.C. Smith. 1994. Derivation of a weighted-average reflection coefficient for mesophyll cell membranes of *Kalanchoë daigremontiana*. *Planta* 193:145–147.
- Nobel, P.S. 1974. Introduction to biophysical plant physiology. W.H. Freeman, San Francisco, CA, 608 p.
- Patrick, J.W. 1997. Phloem unloading: sieve element unloading and post-sieve element transport. *Annu. Rev. Plant Physiol.* 48: 191–222.
- Patrick, J.W. and C.E. Offler. 1996. Post-sieve element transport of photoassimilates in sink regions. *J. Exp. Bot.* 47:1165–1177.
- Quilot, B., M. Génard, J. Kervella and F. Lescourret. 2002. Ecophysiological analysis of genotypic variation in peach fruit growth. *J. Exp. Bot.* 53:1613–1625.
- Reymond, M., B. Muller, A. Leonardi, A. Charcosset and F. Tardieu. 2003. Combining quantitative trait loci analysis and an ecophysiological model to analyze the genetic variability of the responses of maize leaf growth to temperature and water deficit. *Plant Physiol.* 131:664–675.

- Robinson, T.L., A.N. Lakso and S.G. Carpenter. 1991. Canopy development, yield and fruit quality of 'Empire' and 'Delicious' apple trees grown in four orchard production systems for ten years. *J. Am. Soc. Hortic. Sci.* 116:179–197.
- Sansavini, S. 1997. Integrated fruit production in Europe: research and strategies for a sustainable industry. *Sci. Hortic.* 68:25–36.
- Seem, R.C., D.C. Elfving, T.R. Oren and S.P. Eisensmith. 1986. A carbon balance model for apple tree growth and production. *Acta Hortic.* 184:129–137.
- Tardieu, F. 2003. Virtual plants: modeling as a tool for the genomics of tolerance to water deficit. *Trends Plant Sci.* 8:9–14.
- Trewavas, A. 2003. Aspects of plant intelligence. *Ann. Bot.* 92:1–20.
- Trewavas, A. 2004. Aspects of plant intelligence: an answer to Firm. *Ann. Bot.* 93:1353–1357.
- Tubiello, F.N., C. Rosenzweig, R.A. Goldberg, S. Jagtap and J.W. Jones. 2002. Effects of climate change on US crop production: simulation results using two different GCM scenarios. Part I. Wheat, potato, maize and citrus. *Climate Res.* 20:259–270.
- Van Ittersum, M.K., P.A. Leffelaar, H. van Keulen, J. Kropff, L. Bastiaans and J. Goudriaan. 2003. On approaches and applications of the Wageningen crop models. *Eur. J. Agron.* 18:201–234.
- Walcroft, A.S., F. Lescourret, M. Génard, H. Sinoquet, X. Le Roux and N. Donès. 2004. Does variability in shoot carbon assimilation within the tree crown explain variability in peach fruit growth? *Tree Physiol.* 24:313–322.
- Wallach, D., B. Goffinet, J.E. Bergez, P. Debaeke, D. Leenhardt and J.N. Aubertot. 2001. Parameter estimation for crop models: a new approach and application to a corn model. *Agron. J.* 4:757–766.
- Wermelinger, B., J. Baumgärtner and A.P. Gutierrez. 1991. A demographic model of assimilation and allocation of carbon and nitrogen in grapevines. *Ecol. Model.* 53:1–26.
- Wolf, J. 2002. Comparison of two soya bean simulation models under climate change. II. Application of climate change scenarios. *Climate Res.* 20:71–81.
- Yakushiji, H., K. Morinaga and H. Nomami. 1998. Sugar accumulation and partitioning in Satsuma Mandarin tree tissues and fruit in response to drought stress. *J. Am. Soc. Hortic. Sci.* 123:719–726.

Séminaire de restitution de L'ATP « Réserves » (n° 11/2002)
Compte rendu de la réunion: Conclusions & Perspectives
Vendredi 10 novembre (salle 15, 9h00 -12h30)

Etaient présents :

Frédéric Normand (UPR 77, FLHOR), Alexia Prades (UPR 33, CP), Anne Clément-Vidal (UPR59, AMIS), Agnès Guilliot (UMR547 PIAF, Université Blaise Pascal, Clermont-Ferrand), Jean-Pierre Caliman (UPR 34, CP/SMARTRI), Xavier Bonneau (UPR 34, CP), Philippe Thaler (UPR 80, CP), Serge Braconnier (UPR 59, AMIS), Isabelle Mialet-Serra (UPR 59, AMIS).

Objectifs :

- Discuter des acquis et en faire rapidement l'inventaire afin d'envisager à court terme des applications pratiques au champ;
- Envisager des perspectives à cette ATP intra-UPR mais également entre UPRs concernées et intéressées pour poursuivre et continuer à approfondir cette problématique.

Résultats :

Les premières remarques faites portèrent sur:

- L'intérêt à travailler sur des objets très différents afin de couvrir différentes situations de comportements: des fruitiers mais aussi un ligneux comme l'hévéa pour lequel un puits totalement artificiel et créé par l'homme est entretenu ;
- Le fait que parmi les résultats présentés au cours de ce séminaire, nombreux sont ceux qui posent un questionnement fort sur les mécanismes mis en jeu et que nombre d'entre eux doivent être encore analysés pour déboucher sur une théorie cohérente de fonctionnement des quatre espèces étudiées.

Il a été rappelé que deux points importants devraient être dégagés à l'issue de cette ATP :

- Définir des marqueurs ou indicateurs physiologiques (à travers les glucides stockés temporairement par la plante et leurs évolutions au cours du temps), du statut trophique des quatre espèces étudiées, utiles pour un diagnostic sur leur capacité à maintenir des niveaux stables de rendement ou expliquer au contraire des variations de rendement, voire même les prévoir;
- Intégrer l'ensemble des connaissances acquises dans les modèles de fonctionnement en cours de développement afin, à terme, de renforcer leur pouvoir prédictif. Dans un avenir très proche, cela ne sera fait que sur palmier à huile (convention « *EcoPalm* » en cours depuis début 2006 entre les départements CP et AMIS).

① **Les acquis : inventaire de marqueurs par espèce**

Pour ce faire, les résultats les plus marquants par espèce ont été rappelés.

Sur Manguier

L'amidon est le glucide majoritaire dans les compartiments végétatifs ligneux (porte-greffe, tronc, branches charpentières et branches, racines) d'un manguier adulte, le saccharose est majoritaire dans les Unités de Croissance (UC) terminales et sub-terminales qui portent floraison et fructification.

La teneur en saccharose dans les UC terminales et sub-terminales est élevée et peu variable lors du remplissage des fruits. Dans le même temps, la teneur en amidon décroît de façon significative dans les UC porteuses des fruits et dans les racines, et reste stable dans les compartiments végétatifs ligneux aériens anciens (tronc, charpentières, premiers niveaux de ramification des charpentières).

Actuellement, *aucune relation* n'a pu être mise en évidence

- *Avant la floraison*, entre les teneurs en glucides mesurées dans les compartiments végétatifs de la plante et les rendements obtenus;
- *Au moment de la récolte*, entre les teneurs en glucides dans les compartiments végétatifs après le remplissage des fruits et les rendements observés alors.

Sur manguier, il est donc difficile pour l'instant de cibler précisément des marqueurs. Une analyse plus poussée des données doit être menée très prochainement. André Lacoïnte (UMR547 PIAF, INRA, Clermont-Ferrand) fera une mission à la Réunion la semaine du 20 au 24 novembre pour conseiller Frédéric Normand dans cette tâche.

Sur Hévéa

Deux approches ont été mises en œuvre lors de cette étude. La première vise à évaluer le rôle des réserves carbonées lors de la mise en saignée de l'hévéa, la seconde compare les différents systèmes d'exploitation des arbres et notamment le système « DCA » (« double encoche alternée »).

Le saccharose est plus particulièrement présent dans l'écorce alors que l'amidon est majoritaire dans le bois. Celui-ci apparaît comme le marqueur le plus pertinent. Sa variation au cours du temps et en fonction de sa localisation dans le bois semble être en relation avec la production de latex.

Les arbres saignés, qui doivent régénérer le latex exporté ce qui crée un puits artificiel pour les assimilats, accumulent davantage d'amidon que les arbres témoins non saignés. Le système de saignée « DCA » est le système le plus prometteur et le plus productif actuellement (20% de latex produit en plus comparé à un système classique sur les trois premières années de saignée). C'est aussi le système où l'on mesure le plus d'amidon dans le bois du tronc. Une part de la performance de ce système peut être expliquée par l'interaction positive entre les deux encoches de saignée, l'exploitation d'une face de l'arbre conduisant à l'accumulation d'amidon sur la face opposée.

La teneur en amidon, mesurée et suivie au niveau du panneau de saignée, viendra compléter dorénavant le Diagnostic Latex (DL), lors de nouvelles expérimentations. Ce nouveau paramètre pourrait permettre d'évaluer la pertinence de nouveaux systèmes d'exploitation et la performance de différents clones.

Des analyses enzymatiques concernant les principales enzymes impliquées dans les relations sources/puits sont en cours d'études.

Sur Cocotier

Le saccharose est le glucide majoritaire. Cependant, il varie peu voire très peu dans nos conditions de cultures qui sont considérées comme optimales.

L'amidon est un glucide mineur (en faible quantité). En revanche, il a varié très nettement au cours de notre première expérience notamment lors d'une reconstitution des réserves, observée consécutivement à un stress pathologique apparu en début d'expérience puis circonscrit ensuite.

A ce titre, l'amidon pourrait constituer un bon indicateur d'un surplus temporaire en glucides par rapport aux besoins énergétiques de la plante. Il pourrait contribuer ensuite ponctuellement, après hydrolyse, à atteindre à nouveau une productivité optimale. Par ailleurs, la localisation spécifique d'une poche d'amidon dans la zone sous méristématique permettrait de suivre facilement ses variations saisonnières dans le temps et les relier à des événements récurrents ou erratiques (i.e. croissance et production accrues ou l'inverse). Cette hypothèse fondée sur nos premières observations devrait être maintenant vérifiée dans des conditions environnementales plus contraignantes pour cette plante.

Par ailleurs, le rôle « tampon » du compartiment « réserves » a été très clairement établi. En revanche dans nos conditions de culture, les réserves en glucides n'auraient aucun rôle en terme d'impacts sur la production de noix (nombre et biomasse développés), mais cela reste à vérifier dans des conditions plus contraignantes.

Sur Palmier à huile

Le glucose est le glucide majoritaire. L'amidon n'est plus, comme chez le cocotier, un glucide mineur puisqu'il représente 20 % des glucides totaux présents chez un palmier à huile adulte.

A l'échelle de la plante entière, les variations du pool de réserves (phases de mobilisation et de stockage) sont plus intenses chez le palmier à huile que chez le cocotier même dans des conditions optimales de culture. Les réserves carbonées seraient sollicitées de façon plus intense et plus souvent chez le palmier à huile que chez le cocotier. Le déterminisme du stockage / déstockage chez cette *Arecaceae* est encore à rechercher. La situation et les éléments dont nous disposons sont plus complexes et confus que sur cocotier.

Cependant, nous pensons également que l'amidon et ses variations dans le temps pourraient représenter, chez le palmier à huile, un bon indicateur du statut trophique de la plante et de sa productivité à venir. Mais cette hypothèse doit être approfondie.

② Les perspectives envisagées

Un tour de table a été fait à ce sujet.

Sur Manguier

- L'important jeu de données acquis doit être maintenant analysé plus précisément et demandera un certain recul. Ce travail sera initié lors de la mission d'André Lacoïnte (UMR547 PIAF, INRA, Clermont-Ferrand) à la Réunion (définition des méthodes d'analyse des données en fonction des questions posées).
- De nouvelles expériences au champ peuvent être envisagées (perspectives 2007 à 2010) :
 - sur deux variétés aux comportements tranchés en terme d'alternance;
 - avec un suivi des niveaux en carbone dans la plante et de leur impact sur l'induction florale et la fructification sur une durée d'au moins 2 ans dans des conditions contrastées de puits (charges en fruits différenciées) et de sources (ombrages).

- En matière de modélisation, actuellement le modèle biochimique de photosynthèse de Farquhar (travaux antérieurs de Laurent Urban) décrivant l'assimilation en carbone à l'échelle du compartiment foliaire et un modèle de croissance du fruit et d'élaboration de sa qualité (travaux de thèse de Mathieu Léchaudel avec l'adaptation du modèle « *Peach* » (Génard *et al.*)) ont été développés sur manguier. Une approche intégrant l'ensemble des données acquises et ces premiers modèles pourrait être discutée notamment lors de la mission d'André Lacoïnte à la Réunion.
- L'outil « SPIR » (Spectrophotométrie Proche InfraRouge) a été développé sur manguier. Une base de données spectrales, constituée de plus de 2000 spectres, a été acquise dans le cadre de l'ATP. L'analyse de cette base n'a débuté que partiellement sur le compartiment racinaire. Ce travail devrait s'étendre, dans les prochaines semaines, à la calibration de la méthode sur l'ensemble des compartiments décrits. Si la capacité prédictive des calibrations est bonne, cette technique pourrait être utilisée en routine dans le cadre des futures expériences (un appareil est utilisable au CIRAD Réunion), réduisant ainsi les coûts et les temps d'analyses.

En terme de publications et de diffusion des résultats :

Déjà parues

- 1- Damour G., Normand F., 2006. Distribution and variability of dry matter contents in mango tree cv. Cogshall. Communication présentée au 8th *International Symposium on Mango*, 5-10/02/2006, Sun City, South Africa. A paraître dans *Acta Horticulturae*.
- 2- Normand F., Lagier S., Escoute J., Verdeil J-L., Mialet-Serra I., 2006 - Starch localisation in mango tree: histological observations. Communication présentée au 8th *International Symposium on Mango*, 5-10/02/2006, Sun City, South Africa. A paraître dans *Acta Horticulturae*.

En projet

Deux à trois publications sur les sucres de réserve du manguier et leur gestion par la plante sont envisagées.

Une publication sur l'utilisation de la méthode SPIR pour la détermination des teneurs en sucre dans les organes du manguier est également prévue.

Sur Hévéa

L'ATP a permis de répondre à l'essentiel des questions posées en matière de nature, localisation et dynamique des réserves carbonées.

Les perspectives envisagées sont

- L'utilisation du paramètre « amidon » en complément du Diagnostic Latex (DL);
- Une poursuite de l'analyse des données acquises dans le cadre de l'ATP, particulièrement pour passer des données en teneur aux données en quantité (de glucides) par arbre;
- A partir de protocoles allégés, de nouvelles expériences doivent être menées dans des conditions plus contraignantes, sur différents clones (seul un clone a été étudié dans le cadre de l'ATP), afin de suivre également l'impact de la saignée sur la photosynthèse de l'arbre (relation source/puits et existence ou pas d'ajustement);
- Plus spécifiquement, la poursuite des études enzymologiques permettant de mieux cerner les phases de mobilisation et de stockage au niveau du panneau de saignée et leurs intensités, en se focalisant sur les amylases (responsables de l'hydrolyse de l'amidon) et la sucrose synthase (SuSy, associée à la synthèse de l'amidon), sur deux traitements (témoin et système de saignée

« DCA »). Compte tenu des objectifs scientifiques de l'UPR 80, à laquelle appartient maintenant Ph. Thaler, il a été convenu que cet axe de recherche bien qu'intéressant toujours l'UPR 80 sur un plan cognitif, relevait, maintenant, plus des objectifs scientifiques des UPRs 59 & 34, de l'UMR DAP et de l'UMR547 PIAF (Univ. Blaise Pascal – Clermont-Ferrand), et que ces UPRs-UMRs poursuivraient de manière concertée cette activité;

- o Parmi les objectifs initiaux de l'ATP, il s'est avéré que l'étude, par immuno-cytologie, des transporteurs de sucres au niveau des membranes des cellules laticifères, nécessitait la création d'anticorps spécifiques, ce qui sortait du cadre de l'ATP. Cette opération est maintenant poursuivie par l'UMR 547 PIAF, dans le cadre d'un doctorat en collaboration avec l'IRD.

En terme de publications et de diffusion des résultats :

Déjà parues

1- Silpi U., 2004 - Carbon Partitioning in *Hevea brasiliensis* Muell. Arg.: Dynamics among functional sinks (latex regeneration, respiration, growth and reserves) at trunk scale. PhD (Botany). Kasetsart University, Bangkok, Thaïlande.

2- Silpi, U., Lacoïnte, A., Kasemsap, P., Thanysawanyangkura, S., Chantuma, P., Gohet, E., Musigamart, N., Clément, A., Améglio, T. and Thaler, Ph., 2007 – Carbohydrate reserves as a competing sink: Evidence from tapping the rubber tree. *Tree Physiology*. Acceptée.

3- Chantuma P. 2006 - Dynamics of carbohydrate reserves as related to tapping in rubber tree. PhD (Botany). Kasetsart University, Bangkok, Thaïlande.

En projet

Deux articles supplémentaires seront extraits de la thèse de Pisamai Chantuma (RRIT) :

4- Chantuma P., Lacoïnte A., Kasemsap P., Thanisawanyangkura S., Gohet E., Clément A., Améglio T. and Thaler P. 2007 - Carbohydrate reserves as a competing sink: evidence from tapping the rubber tree. II Relationship with latex yield and latex metabolism. A soumettre à *Tree Physiology*.

5- Chantuma P., Lacoïnte A., Kasemsap P., Thanisawanyangkura S., Gohet E., Améglio T., Thaler P., 2007 - Physiological basis of the Double Cut Alternative tapping system. A soumettre à *Journal of Rubber Research* ou *Indian Journal of Rubber Research*.

Sur Cocotier et sur Palmier à huile

Il apparaît maintenant évident que des conditions de culture constamment favorables limitent l'explication du rôle des réserves pour ces deux *Arecaceae*, et limitent les études faites sur le compartiment fructifère et l'élaboration de sa qualité (actuellement pour le cocotier). La consolidation des hypothèses avancées (amidon, indicateur physiologique d'un surplus ; ajustement de l'offre directe en carbone (photosynthèse) sur les demandes énergétiques de la plante entière, pas de relations simples entre teneurs en glucides entre une feuille et le régime qu'elle axile...) suppose que l'on se place maintenant dans des conditions de contraintes (hydriques notamment) marquées et d'occurrences assez régulières.

Par ailleurs, ces études n'ont porté que sur un écotype. Des comparaisons de matériels végétaux présentant des morphologies très tranchées (i.e. taille du stipe ; comparaisons de Grands, de Nains et d'hybrides pour le cocotier, de différentes lignées pour le palmier à huile) permettraient d'affiner le ou les schéma(s) de fonctionnement proposé(s).

Concrètement cela passerait

- o par une simplification des méthodes d'investigations utilisées et la mise au point d'un modèle performant permettant de proposer des pronostics fiables ;

Mais aussi,

- par une validation, à travers des mesures de photosynthèse à l'échelle foliaire, de l'hypothèse de rétroactions entre la source photosynthétique et la demande mise en évidence dans le cadre de cette étude, en réalisant des mesures sur plusieurs traitements (i.e. ablation de tous les régimes avant fécondation ; élagages de feuilles (d'intensité moyenne à forte) ;
- par un suivi, parallèlement aux teneurs en glucides dans les principaux compartiments de stockage et à leur évolution, des transferts de carbone entre organes sources et puits. Deux approches pourraient être alors utilisées :
 - un marquage isotopique (au ^{13}C , isotope stable ou froid), permettant de suivre les mises en réserves et les mobilisations mais aussi de discriminer précisément la part de carbone allouée au puits venant des réserves de celle néo-synthétisée (photosynthèse) ;
 - une étude enzymatique en analysant les activités d'enzymes clés du métabolisme des glucides, associées à des changements d'état mais aussi à des flux de carbone plus ou moins intenses et reflétant la force des puits de stockage ou d'utilisation des puits dans lesquelles elles sont recherchées.

Un modèle de fonctionnement des *Arecaceae*, EcoPalm (Combres *et al.*, 2003 ; cf. ces proceedings) est en cours de développement sur palmier à huile. Cet outil a pour objectif la prévision saisonnière des récoltes en fonction du climat et, à terme, du génotype. *Les informations acquises dans le cadre de ce travail permettraient d'affiner les concepts à prendre en compte pour décrire la gestion du carbone néo-synthétisé et stocké temporairement par la plante, mais, également, de développer des prévisions non seulement en terme de quantités de fruits produits (nombre et biomasse produites) mais aussi en terme de qualité (études réalisées par Alexia Prades).*

Pour le cocotier, actuellement, se pose le problème crucial du site et des partenaires encore non identifiés pour développer à court ou moyen termes les perspectives envisagées. Une réflexion sur les solutions possibles sera amorcée début janvier 2007 (réunion organisée par Bertrand Tailliez, chargé de mission « Palmier – Cocotier »).

*Pour le palmier à huile, les perspectives citées ci-dessus sont et seront développées dans le cadre d'une convention « EcoPalm » passée entre les départements CP et AMIS, et accueillie sur le terrain par SMARTRI, l'institut de recherche de PT Smart Tbk (Indonésie). L'objectif principal de cette convention est de développer un modèle simple, capable de simuler, sur la totalité du cycle de culture d'une parcelle de palmier à huile, les variations saisonnières et interannuelles de production. Cet outil devrait servir à la prévision des récoltes (à court et à moyen termes) et être testé pour l'aide à la décision (choix des sites d'implantations, des matériels végétaux les mieux adaptés, de la densité de plantation...). Pour ce faire, un volet porte sur l'amélioration du bilan carboné du modèle *EcoPalm*, en prenant en compte explicitement le pool de réserves carbonées mais aussi l'allocation de carbone au compartiment fructifère et la synthèse d'huile. Dans l'optique globale d'une amélioration de ce bilan carboné,*

- Un PhD a débuté, il y a un an, ayant pour thème « *Analyse et modélisation des interactions entre la phénologie et la gestion du carbone chez le palmier à huile : influence des paramètres génotypiques sur l'adaptabilité et la productivité* ». Mesures de photosynthèse, suivis des stocks de glucides dans la plante parallèlement aux croissances végétative et fructifère, recherches d'enzymes clés sont réalisés sur deux lignées, deux sites (i.e. avec et sans contraintes hydriques), et deux traitements (i.e. témoin et ablation systématique des régimes avant fécondation).
- *Une étude architecturale* suivie de la création de maquettes numériques en 3D est envisagée. L'intérêt de cette étude réside dans la possibilité de réaliser ensuite sur les maquettes créées des calculs d'interception du rayonnement et de photosynthèse à une échelle fine d'espace

(échelle de la foliole) et de temps (infra-horaire), en utilisant des méthodes numériques d'intégration sans faire appel à des hypothèses simplificatrices très souvent sources d'erreurs lors du passage à la plante entière sur la journée. Cette approche que nous considérons comme de référence, sera utilisée pour valider l'approche « big-leaf » simplificatrice développée dans le modèle, *EcoPalm*. De plus, les données utilisées pour modéliser l'architecture des arbres, seront également analysées pour définir des indicateurs de l'effet des conditions de culture sur la croissance des arbres. L'étude de l'évolution des paramètres architecturaux permettra ensuite de représenter les arbres à différents stades de croissance et en fonction des conditions hydriques. De multiples expériences de simulation deviendront alors possibles à moindre coût. Ce volet pourrait être développé en partenariat avec l'UMR TETIS et notamment l'UPR 60 (Geotrop avec Camille Lelong notamment).

- Une étude sur la maturation du régime et les taux d'huile synthétisée au cours du développement du régime, en fonction des saisons et des lignées devrait être mise en place très prochainement et faire l'objet d'une formation diplômante pour le chercheur indonésien en charge de cette activité, M. Fahri Siregar (SMARTRI). Le montage scientifique et administratif de ce dossier est en cours.
- Le marquage du carbone à l'aide d'un isotope stable (type ^{13}C) apporterait des informations complémentaires sur les flux de carbone dans la plante, les pools de carbone mobilisés et la force des puits en présence. Ce volet pourrait être développé en relation avec le centre de recherche indonésien Batan.
- Enfin l'approche « SPIR » développée sur cocotier dans le cadre de l'ATP, serait à développer sur palmier à huile. Les valeurs de référence (du laboratoire) étant déjà acquises, le développement de cet outil pourrait être réalisé à moyen terme.

En terme de publications des résultats et de diffusion des résultats:

Déjà parues

1- Mialet-Serra, I., 2005 – Rôle et gestion des réserves carbonées face à la variabilité du climat chez une monocotylédone arborescente, le cocotier (*Cocos nucifera* L.) : Analyse et bilan. Th. : Ecophysiologie : ENSA de Montpellier, 112 p. + 6 annexes.

2- Mialet-Serra I., Clément A., Sonderegger N., Roupsard O., Jourdan C., Labouisse J-P. & Dingkuhn M., 2005 - Assimilate storage in vegetative organs of coconut (*Cocos nucifera* L.). *Experimental Agriculture*, 41 (2): 161-174.

En projet

3- Davrieux, F., Mialet-Serra, I., Clément-Vidal, A., Prades, A.– NIRS : a good tool to estimate sugar contents of vegetative organs of coconut palm and their variations or not ? à soumettre dans *Journal of NIRS*.

4- Legros, S., Mialet-Serra, I., Caliman, J-P., Clément-Vidal, A., Siregar, F., Widiastuti, L., Jourdan, C., & Dingkuhn, M., 2007 – Nature, Distribution and Seasonal changes of carbohydrates reserves in adult oil palm tree: a comparison with adult coconut palms.

5- Mialet-Serra, I., Clément-Vidal, A., Roupsard, O., Sonderegger, N., Flori, A., Jourdan, C., Labouisse, J-P., Dingkuhn, M.– Role of carbon reserves in an arborescent monocot, the coconut palm (*Cocos nucifera* L.) in the face of climate variability : Analysis and balance. à soumettre à *Functional Plant Biology*.

6- Mialet-Serra, I., Prades, A., Labouisse, J-P., Clément-Vidal, A., Dingkuhn, M. - Physical and chemical characters of the coconut (*Cocos nucifera* L.) growing « leaf-bunch phytomer »: dependence or not on climate? Plusieurs revues sont ciblées.

7- Mialet-Serra, I., Croisetu, M., Fougerolles, T., Escoute, J., Verdeil, J-L., Caliman, J-P., Legros, S., Clément-Vidal, A. and Dingkuhn, M. – *In situ* starch localisation in *Cocos nucifera* L. and *Elaeis guineensis* Jacq. : The major reserve carbohydrate in these *Arecaceae* or not? à soumettre à *Plant, Cell and Environment*.

8- Prades, A., Assa, R., Mialet-Serra, I., Labouisse, J-P., Clément-Vidal, A., Davrieux, F., Konan, J-L., Sileye, T., and Rouzière A.– Quality Assessment of Coconut (*Cocos nucifera* L.) Fruits from two Hybrids at Different Stages of Maturity, à soumettre. Plusieurs revues telles que *Plant Science*, *Plant Physiology and Biochemistry*, *Field Crops Research* sont ciblées.

9- Prades, A., Davrieux, F., Mialet-Serra, I., Assa, R. R., Rouzière, A. - NIRS: a new approach for estimating coconut (*Cocos nucifera* L.) fruit quality during maturation. à soumettre dans *Journal of NIRS*.

Novembre 2006.

ATP « Reserves » (n° 11/2002)
Listing of publications, communications & training reports

Publications in reviews with impact factors

Mialet-Serra I., Clément A., Sonderegger N., Roupsard O., Jourdan C., Labouisse J-P. & Dingkuhn M., 2005 - Assimilate storage in vegetative organs of coconut (*Cocos nucifera* L.). *Experimental Agriculture*, 41 (2): 161-174.

Silpi U., Lacoïnte A., Kasemsap P., Thanisawanyangkura S., Chantuma P., Gohet E., Musigamart N., Clément A., Améglio T. and Thaler P. 2006 - Carbohydrate reserves as an active sink: evidence from tapping the rubber tree. Accepted in *Tree Physiology*.

Publications in reviews without impact factors

Chantuma P., Thanisawanyangkura S., Kasemsap P., Gohet E. and Thaler P. 2006. Distribution patterns of latex sucrose content and concurrent metabolic activity at the trunk level with different tapping systems and in latex production bark of *Hevea brasiliensis*. *Kasetsart Journal, Natural Sciences* 40 (3). (Accepted).

Silpi U., Chantuma P., Kasemsap P., Thaler P., Thanisawanyangkura S., Lacoïnte A., Améglio T. and Gohet E. 2006 - Sucrose and Metabolism Distribution Patterns in the Latices of Three *Hevea brasiliensis* Clones: Effects of Tapping and Stimulation on the Tree Trunk. *Journal of Rubber Research* 9 (2) 115-131.

Drafts of publications to submitted to reviews with impact factors

On **MANGO**, two or three publications on sugars reserve and their management by the plant are considered. A publication on the use of NIRS method for the determination of the sugar concentrations in the vegetative and fruit organs is also envisaged.

On **RUBBER**, **OIL PALM** and **COCONUT** palms:

Chantuma P., Lacoïnte A., Kasemsap P., Thanisawanyangkura S., Gohet E., Clément A., Améglio T. and Thaler P. 2007 - Carbohydrate reserves as a competing sink: evidence from tapping the rubber tree. II Relationship with latex yield and latex metabolism. *Tree Physiology* is targeted.

Chantuma P., Lacoïnte A., Kasemsap P., Thanisawanyangkura S., Gohet E., Améglio T., Thaler P., 2007 - Physiological basis of the Double Cut Alternative tapping system. *Journal of Rubber Research* or *Indian Journal of Rubber Research* are targeted.

Davrieux, F., Mialet-Serra, I., Clément-Vidal, A., Prades, A., 2006 – NIRS : a good tool to estimate sugar contents of vegetative organs of coconut palm and their variations or not ? *Journal of NIRS* is targeted.

Legros, S., Mialet-Serra, I., Caliman, J-P., Clément-Vidal, A., Siregar, F., Widiastuti, L., Jourdan, C., & Dingkuhn, M., 2007 – Nature, Distribution and Seasonal changes of carbohydrates reserves in adult oil palm tree: a comparison with adult coconut palms.

Mialet-Serra, I., Clément-Vidal, A., Roupsard, O., Sonderegger, N., Flori, A., Jourdan, C., Labouisse, J-P., Dingkuhn, M., 2006 – Role of carbon reserves in an arborescent monocot, the coconut palm (*Cocos nucifera* L.) in the face of climate variability : Analysis and balance. *Functional Plant Biology* is targeted.

Mialet-Serra, I., Prades, A., Labouisse, J-P., Clément-Vidal, A., Dingkuhn, M., 2006 - Physical and chemical characters of the coconut (*Cocos nucifera* L.) growing « leaf-bunch phytomer »: dependence or not on climate? Several reviews are targeted.

Mialet-Serra, I., Croisetu, M., Fougerolles, T., Escoute, J., Verdeil, J-L., Caliman, J-P., Legros, S., Clément-Vidal, A. and Dingkuhn, M., 2006 – *In situ* starch localisation in *Cocos nucifera* L. and *Elaeis guineensis* Jacq. : The major reserve carbohydrate in these *Areaceae* or not? *Plant, Cell and Environment* is targeted.

Prades, A., Assa, R., Mialet-Serra, I., Labouisse, J-P., Clément-Vidal, A., Davrieux, F., Konan, J-L., Sileye, T., and Rouzière A., 2006 – Quality Assessment of Coconut (*Cocos nucifera* L.) Fruits from two Hybrids at Different Stages of Maturity, à soumettre. *Plant Science, Plant Physiology and Biochemistry*, or *Field Crops Research* are targeted..

Prades, A., Davrieux, F., Mialet-Serra, I., Assa, R. R., Rouzière, A., 2006 - NIRS: a new approach for estimating coconut (*Cocos nucifera* L.) fruit quality during maturation. *Journal of NIRS* is targeted.

PhD

Chantuma P. 2006 - Dynamics of carbohydrate reserves as related to tapping in rubber tree. PhD (Botany). Kasetsart University, Bangkok, Thaïlande.

Mialet-Serra, I., 2005 – Rôle et gestion des réserves carbonées face à la variabilité du climat chez une monocotylédone arborescente, le cocotier (*Cocos nucifera* L.) : Analyse et bilan.Th. : Ecophysiologie : ENSA de Montpellier, 112 p. + 6 annexes.

Silpi U., 2004 - Carbon Partitioning in *Hevea brasiliensis* Muell. Arg.: Dynamics among functional sinks (latex regeneration, respiration, growth and reserves) at trunk scale. PhD (Botany). Kasetsart University, Bangkok, Thaïlande. ISBN 974-274-434-3.

Conferences

- Chantuma, A, Thaler Ph., Chantuma, P. and Gohet, E. 2003 - Estimation of the biomass of three rubber clones with different architectures. Rubber Wood, Cropping and Research. Regional Workshop. Kasetsart University, Bangkok, Thailand. 12-14 November 2003.
- Chantuma P., Thaler P., Kasemsap P., Thanisawaniangkura S. and Lacoite A. 2006. Dynamics of carbohydrate reserves in trunk of rubber trees as related to tapping. Thai-French Rubber seminar, Bangkok, Thailand, 1-2 June 2006.
- Chantuma P., Thaler P., Gohet E., Thanisawaniangkura S. and Kasemsap P. 2006. Carbohydrate distribution at trunk level of *Hevea brasiliensis*. International Natural Rubber Conference, Ho Chi Minh City, Vietnam, 13-14 November 2006.
- Damour, G., Normand, F., 2006 - Distribution and variability of dry matter contents in mango tree cv. Cogshall. Communication présentée au 8th *International Symposium on Mango*, 5-10/02/2006, Sun City, South Africa. A paraître dans *Acta Horticulturae*.
- Davrieux, F., Prades, A., Mialet-Serra, I., Dupuis, S., Assa, R., 2005 - Physico-Chemical Changes in the Fruits of Two Coconut (*Cocos nucifera* L.) Hybrids during Ripening. A NIRS-boosted Study. Frutic'05, Informations and Technologies for sustainable fruit and vegetable production, Montpellier, 12-16 septembre 2005. Poster.
- Legros, S., Mialet-Serra, I., Caliman, J-P., Clément-Vidal, A., Siregar, F., Widiastuti, L., Jourdan, C. and Dingkuhn, M., 2006 – Carbohydrates reserves in 9 years old oil palm: Nature, Distribution and Seasonal changes, Communication IOPC 2006, Bali, 19 – 23 June 2006.
- Mialet-Serra, I., Clément, A., Sonderegger, N., Roupsard, O., Flori, A., Jourdan, C., Labouisse, J-P., Dingkhun, M., 2005 – Assimilate storage in vegetative organs of coconut. International Forum of Coconut, Cairns (Nord Queensland, Australie), 22 – 24 novembre 2005. Poster.
- Normand F., Lagier S., Escoute J., Verdeil J-L., Mialet-Serra I., 2006 - Starch localisation in mango tree: histological observations. Communication présentée au 8th *International Symposium on Mango*, 5-10/02/2006, Sun City, South Africa. A paraître dans *Acta Horticulturae*.
- Normand, F., Damour, G., Cabeu, P., Dechazal, M., Jourdan, C., Jannoyer, M., 2004 - Teneurs en matière sèche et répartition de la biomasse chez le manguier. Communication présentée à la Réunion Annuelle du département FLHOR du Cirad, 5-9/07/2004, Montpellier.
- Normand F., Jourdan C., 2004 - Le système racinaire du manguier à la Réunion, biomasse et dynamique racinaire. Communication présentée à la Réunion Annuelle du département FLHOR du CIRAD, 5-9/07/2004, Montpellier.
- Prades, A., Davrieux, F., Mialet-Serra, I., Assa, R., Rouzière, A., 2005 – Rapid diagnosis of coconut fruit quality and sustainable small-scale processing, International Forum of Coconut, Cairns (Nord Queensland, Australie), 22 – 24 novembre 2005. Poster.

Training reports

- Annajdaoui, Hanana, 2003 - Caractérisation immunologique de protéines membranaires chez l'hévéa (*Hevea brasiliensis*) - Mémoire de stage – 15 p. Accueil Université Blaise Pascal, Clermont-Ferrand.
- Chantuma, Pisamai, 2004 - Hydrolysis of starch reserves and the export of soluble sugars in Hevea - Université Blaise Pascal, Clermont-Ferrand. ATP training report.
- Croisetu, Murielle, 2003 - Anatomie des différents organes du cocotier (*Cocos nucifera* L.) et localisation des réserves carbonées. Mémoire de stage. Maîtrise de Biologie Cellulaire et Physiologie Option Physiologie Végétale Appliquée. 51 p.
- Damour, Gaëlle, 2003 - Le manguier (*Mangifera indica*) 'Lirfa' à la Réunion. Étude de la matière sèche de l'arbre et des réserves carbonées du système racinaire : teneurs et répartition. Mémoire de stage de césure INA-PG, 60 p + annexes.
- Dechazal, Magali, 2003 - Étude du système racinaire et estimation des réserves carbonées souterraines chez le manguier adulte à la Réunion. Mémoire de stage ISTOM, 74 p + annexes.
- Deveau, Aurélie, 2003 - Étude de la photosynthèse du cocotier : paramétrage du modèle de photosynthèse de Farquhar à l'aide de mesures à l'échelle foliaire. Modélisation de la photosynthèse à l'échelle de la canopée- Mémoire de stage. Magistère Inter-Universitaire Paris 6, 7, Sud et ENS Biologie-Biochimie. 22 p.
- Dupuis, Stéphane, 2004 – Contribution à la mise au point d'une méthode de mesure par proche infrarouge (SPIR) de la qualité de la noix de coco en vue de la compréhension et du suivi de la maturation des fruits. Mémoire de stage. DESS « perfectionnement en analyses chimiques et spectroscopique ». 31 p. + annexes.
- Fougerolles, Thomas, 2004 – Anatomie des différents organes du palmier à huile (*Elaeis guineensis*) et localisation des réserves amylacées. Mémoire de stage de Maîtrise de biologie cellulaire mention physiologie végétale appliquée. Université Montpellier II, 26 p. + annexes.
- Kansou, Kamal, 2003 – Structure et biomasse du palmier à huile (*Elaeis guineensis*) : Résultats préliminaires – Mémoire de stage de césure, ENSAR, 60 p. + annexes. (french and english versions).
- Lagier, Sébastien, 2003 - Étude histo-cytologique des réserves carbonées chez *Mangifera indica* L. variété Lirfa. Mémoire de maîtrise, Université Montpellier II, 26 p + annexes.
- Legros, Sandrine, 2005 - Répartition et dynamique des réserves carbonées dans les compartiments aériens du manguier entre le repos avant floraison et la fin de la récolte. Relations avec sa charge en fruits. Mémoire de fin d'études, ENITA, Clermont Ferrand, 43 p + annexes.
- Puccinelli, Delphine, 2003 - Mise au point d'une méthode rapide d'extraction de lipides à partir de l'amande de coco. Mémoire de stage. Institut Universitaire et Technologique de Montpellier. 46 p.

Sayag, Samuel, 2004 – Construction de la base de données « ReservePalm » (1^{ère} partie). Mémoire de stage de 1^{ère} année ENITA de Bordeaux. 24 p. + annexes.

Siregar, Fahri, 2006– Analysis of vegetative and fruit growths of adult oil palm: seasonal changes and applied treatments effects, ATP training report. 7p. (in press).

