Progress Report 1

Period 21st February 03 - 21st September 03

Oil Palm Ecophysiology

Carbon isotope discrimination

Aek Pancur, IOPRI, May 03

Phenology and water balance

Inco projects and agro-model platform

Dr Emmanuelle Lamade

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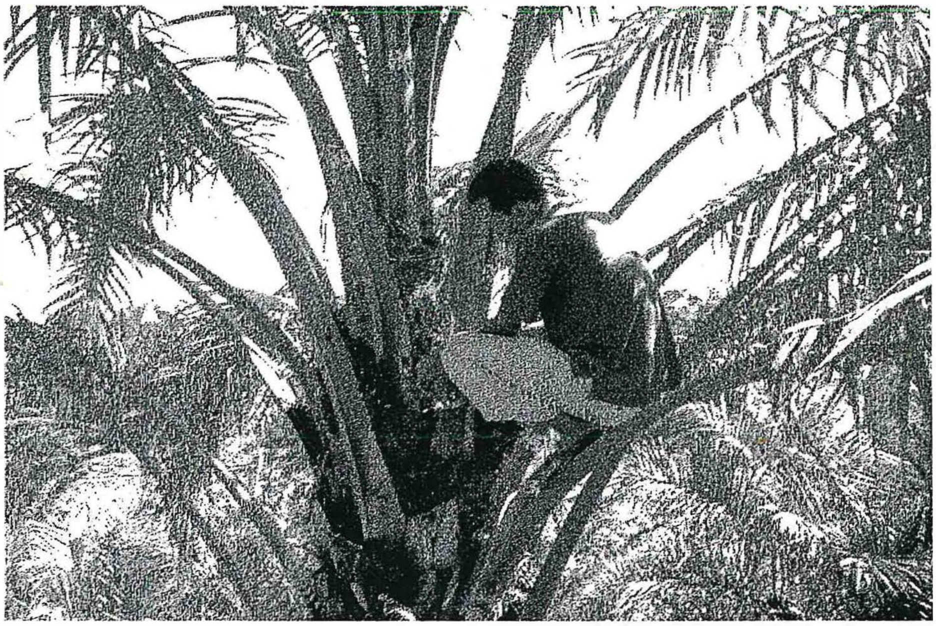


Aek Pancur, IOPRI, May 03

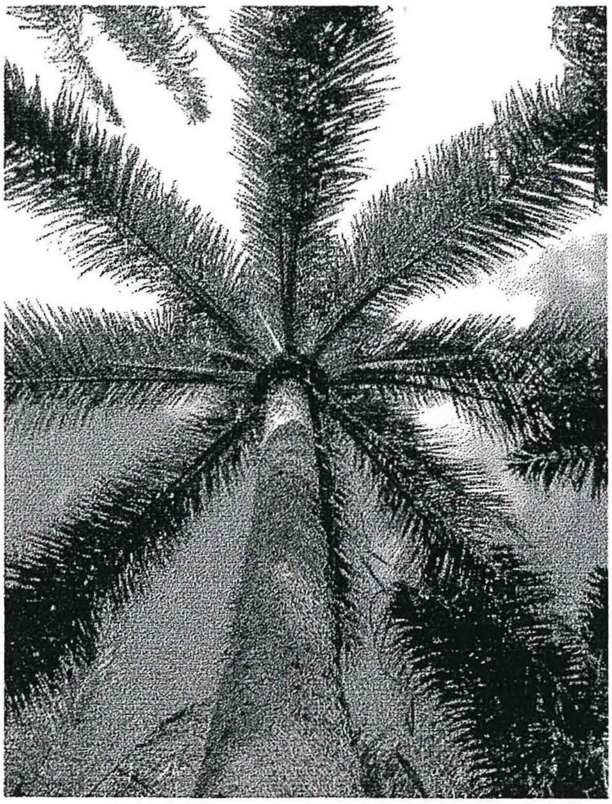
Phenology and water balance

Inco projects and agro-model platform

Dr Emmanuelle Lamade

Preface

This report intends to give a general grid of the on going work on oil palm ecophysiology in IOPRI and to offer new prospectives in the light of the important role of ecology nowadays in the agricultural context of this tropical tree crop...



"Father tree" La Mé

Medan, 7th November 2003

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Introduction

Following the research proposals already elaborated in 2001 (cf. document 1 send to IOPRI in 2001: "Carbon isotope discrimination: a method to investigate: (1) the transpiration yield of the dry matter production and (2) the use of the reserves in oil palm") about the use of carbon isotope discrimination to investigate metabolism processes involved in the phenological development of oil palm, first, collecting samples have been done in Aek Pancur on the clone MK60. Because, it is the first time that this method will be use on oil palm to identify biomass allocation pathway (other similar studies have been done by Arndt and Wanek on tropical trees in 2002), only three aspects will be achieved in this first step. The first one is concerning the growth pattern of the leaves and especially the precise identification of the stage when each leaf is getting autrotrophy from heterotrophy (expected around rank 1 of course). The second aspect is dealing with the evolution of the starch composition of the trunk during all its growth to investigate the possible effect of climatic conditions on the elaboration of possible carbohydrate reserves. The third particularly focused on the oleosynthesis process from pollination to the maturation of each fruit. The best investigation will be to follow the metabolism involved in the process of the transformation of sugar to oil in the fruit during 4 or 5 months. This constitutes a new research for oil palm.

The second part of the ecophysiological new research joint programme between IOPRI , CIRAD and IRD is facing the challenge to propose a water balance model, based on the PhD Thesis's result of Dr Hasril Hasan (2003) which could explained phenological variation of oil palm along an ecological gradient scale from the North of

Sumatra (Kebun Baru, 04°29'N, Aceh) to the South (Rejosari, 5°19'S). Maybe, the existing model of Shuttelworth and Wallace (1985) which include two transpiration levels (soil and vegetation) which is an extension of the classic model of Penman-Monteith (1965) could be chosen. Stomatal resistance of the canopy, soil resistance as well as energy balance between soil and vegetation will be measured and collected from pertinent oil palm substations. A new experimental design will be propose in the next report involving the comparison of the Bowen ratio method and the Jarvis'model.

The third part of this report is constituted by two INCO project proposals, one from CIRAD (Co-ordinator: Dr F. Enjalric) about the elaboration of new agro-environmental indicators for agroforestry cropping system, the second by TTZ Bremerhaven-Environmental Institute about sustainability-oriented management for oil palm production in south-east Asia. These two projects are reflecting the current official status of sustainability policy as far as tree crops are concerned in South - East Asia.

To conclude a short overview of the platform model WaNuLCAS 2.1 (model for Water Nutrient Light Capture Agroforestry Systems), elaborated by M. van Noordwijk and B. Lusiana (2000) (ICRAF, Bogor) gives a good example of a complex model (MAS: multi-agent system) in which an oil palm model based on phenology may be included to investigate other exogene factors (fertiliser, weeding...) effect upon yield.

Part 1. Carbon isotope discrimination

1. Carbon isotopic discrimination: what for?

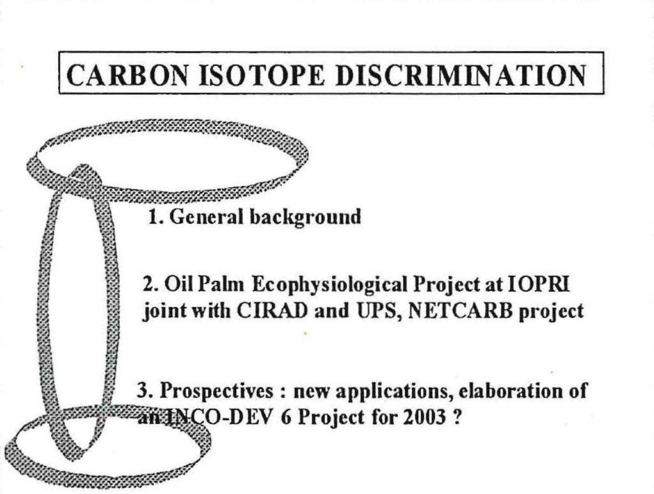
(Total restitution of an UPS training course about isotope written by Jaleh Ghashghaie, Guillaume Tcherkez and Gabriel Cornic)

UPS's Team isotope (Laboratory of Prof. B. Saugier)
-**Dr Jaleh Ghashghaie, University of Paris XI (Orsay):**
Co-ordinator of the European project NETCARB, isotopic discrimination,
- **Prof Gabriel Cornic, University of Paris XI (Orsay) :**
photosynthesis and environmental constraints.
- **Ing. Caroline Lelarge** : Specialist in isotopic mass spectrometry (IBP, Orsay).
- **Guillaume Terchez PhD student University of Paris XI (Orsay)**
- **Dr Salvator Nogues, Researcher**

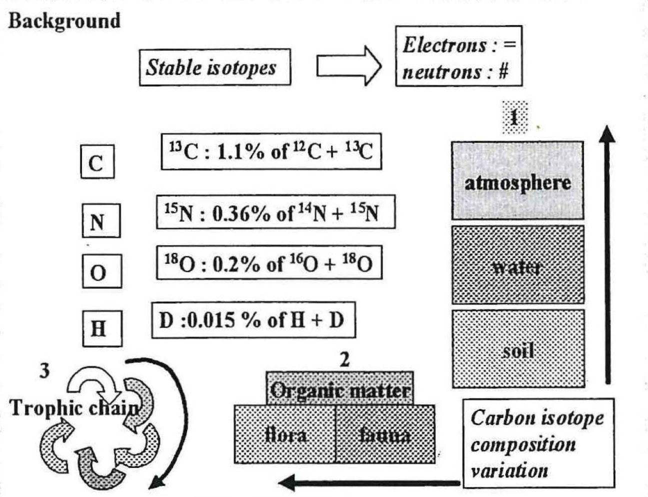
The main elements in Biology (C,N,O,H...), as others elements do exist under stable isotope forms which present same number of electron but are different with the number of neutron.

DIAP 2
It can be distinguished two kinds of isotope: « light » (less neutrons) and « heavy ». Light isotopes are better spread whereas « heavy » can be only detected. But it still possible to measure precisely their quantity. It is the case of elements as:

- CARBONE ^{13}C : 1.1% of ^{12}C+^{13}C
- NITROGEN ^{15}N : 0.36 % of ^{14}N+^{15}N
- OXYGEN ^{18}O : 0.2% of ^{16}O +^{18}O
- HYDROGEN D: 0.015 % of H+D



Diap. 1



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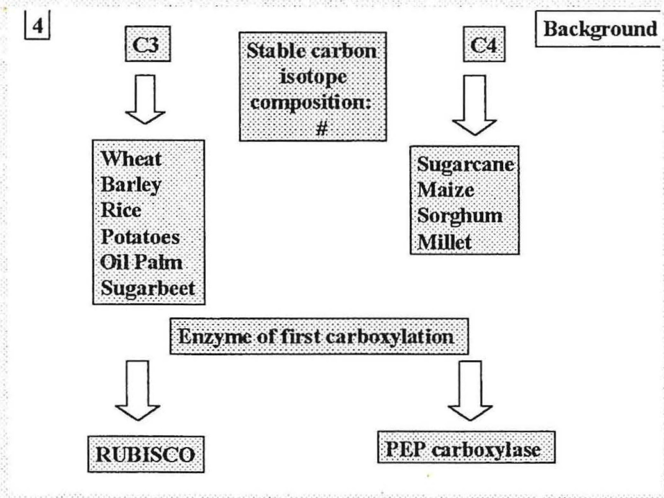

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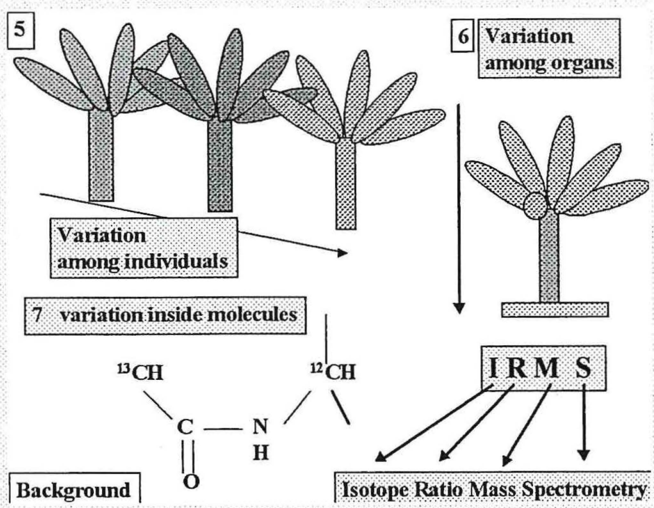

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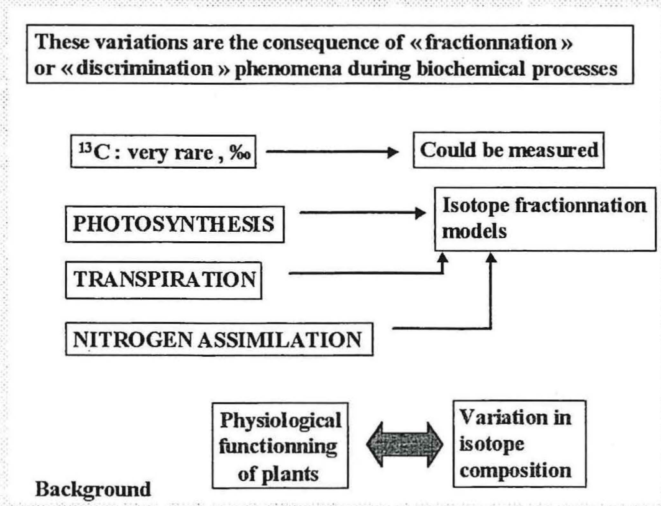

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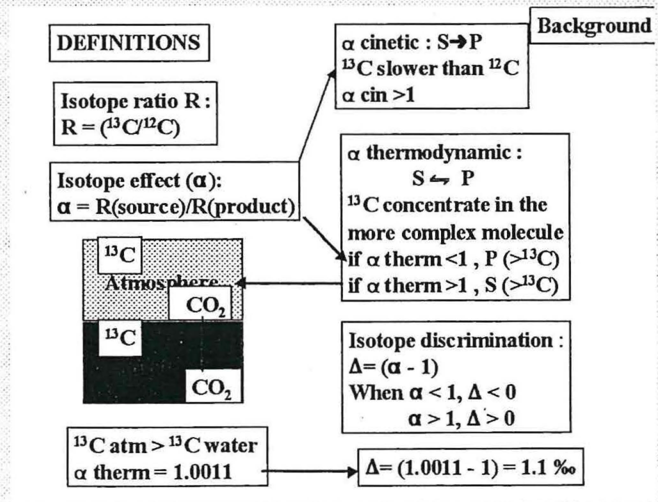


Diap.6

DIAP 3, DIAP 4

The quantity of natural isotopes is variable in the different compartment of the biosphere (air, organic matter from plants or animals), between C3 and C4 plants, between the different trophic levels, individuals, organs, metabolic fraction, and even for a same molecule within the atoms.

These differences are, of course, very small, but may be still measurable with a mass **spectrometer**.

DIAP 5

Natural variations are due to the phenomena of isotope « fractionation » or «discrimination » during molecular transformation, for example between $^{13}CO_2$ and $^{12}CO_2$ during diffusion or enzymatic reaction.

The difference in isotope mass is modifying physical properties, as a consequence, during enzymatic reaction, the isotopic ratio of the final product will be different of the starting one.

The fractionation or discrimination can be evaluated by the variation in the isotopic composition before and after its transformation.

For the main important physiological process, for example photosynthesis, transpiration, nitrogen assimilation, the development of models that described specific fractionation have been done. This can explained the variation in the isotopic ratio in relation with the physiological functioning of the plants.

DIAP 6

b. Definitions

-Isotope ratio:

for the carbon ,

$$R = (^{13}C/^{12}C)$$

variable from one to another organic component.

-Isotope effect:

the isotopic effect (α) is happened during the transformation of one subtract (S) in a product (P):

$$S \longrightarrow P$$

And can be defined as the ratio between the R of the source (S) and that of the product:

$$\alpha = R\ (\text{source})/R\ (\text{product}) \qquad (1)$$

It can be distinguished kinetic isotope effects and thermodynamic isotope effects:

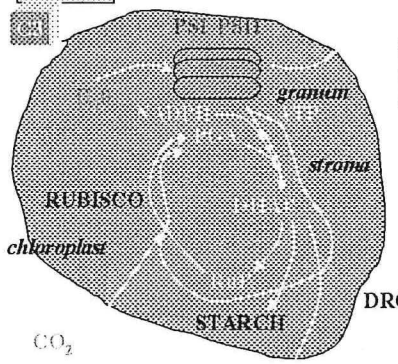
The kinetic isotope effect: related to variation in the kinetic reaction depending on mass. During the transformation of S to P, heavy isotope ^{13}C will react slower than light ^{12}C; in consequence, P product will contains less ^{13}C than S.

The isotopic thermodynamic effects happen during reactions at equilibrium. During equilibrium

$$S \rightleftarrows P$$

The heavy isotope will concentrate in the more stable subtract, in which it will have more molecular link. If α therm< 1, P will contains more heavy isotope than S. It is the

CALVIN CYCLE



PHOTOSYNTHETATES

Isotope effect on the carboxylation by the Rubisco

In vitro : no limitation of CO_2

$\alpha = 1.0309$
$\Delta = (1.0309 - 1)$
$\Delta = 30.9$ ‰

In vivo :(i) limitation of the CO_2 diffusion by stomata

DROUGHT → $\Delta = 4.4$ ‰

(ii) limitation by Rubisco :

$\Delta = 30.0$ ‰

Background

Diap. 7

Isotope Composition

INTERNATIONAL STANDART :
$\delta\ ^{13}C = [(Rsample/Rstandard)-1]$

For the carbon : a calcareaous fossil - Belemnite , PEE DEE stone from South Carolina (PDB), very rich in ^{13}C : R = 0.0112372

For Nitrogen : atmospheric N_2
For Oxygen and Hydrogen : oceanic water

Examples:
- R $^{13}C/^{12}C$ CO_2 atmospheric =0.0111473; $\delta\ ^{13}C$ CO_2 air = -8 ‰
- R $^{13}C/^{12}C$ C3 plants = 0 .0109338; $\delta\ ^{13}C$ C3 = -27 ‰
- R $^{13}C/^{12}C$ C4 plants = 0.0111136; $\delta\ ^{13}C$ C4 = -11 ‰

Isotope discrimination

Δ C3 ~(-8 ‰)-(-27 ‰) = 19 ‰

$\Delta = (\delta\ ^{13}C\ source - \delta\ ^{13}C\ product) / (1 + \delta\ ^{13}C\ product)$

$\Delta \sim \delta\ ^{13}C\ source - \delta\ ^{13}C\ product$

Background

Diap.8

Examples of application of carbon isotope

Background

1. Isotope discrimination against ^{13}C for the C3 plant during photosynthetic assimilation : model of Farquhar et al. (1982) :

$\Delta = a * [(pa-pi)/pa] + b * pi/pa$
$\Delta = a + (b - a) * (pi-pa)$

with pi as partial pressure of CO_2 in intercellular space
and pa as partial pressure of CO_2 in atmosphere
(pi, pa physiological parameters under dependance of stomata and carboxylation rate)
a: diffusion parameter
b: carboxylation parameter
a, b isotope parameters

Application - at instantaneaous scale // leaf gas exchange
- at daily scale : photosynthetate products (soluble sugars)
- at intregrated scale : leaf organic matter

Diap. 9

Background

2. Measurement of the mesophyl resistance : model of Evans et al. 1986.

Correlation between SLW /Δ with different wheat 's genotypes and drought resistance (Merah, 1999)

3. Discrimination against ^{13}C for C4 plants

The Δ for C4 plants is less than for C3 plants :
C4 : Δ =3-4 ‰ , C3 = 19-20 ‰

4. Relation between isotope discrimination and WUE for C3 plants : drought resistance indicator ?

Diap. 10

WUE : production of biomass/water comsumption

Δ

Indicator of plant adaptation to drought condition

Indirect evaluation of WUE Farquhar et al., Ehleringer 1991

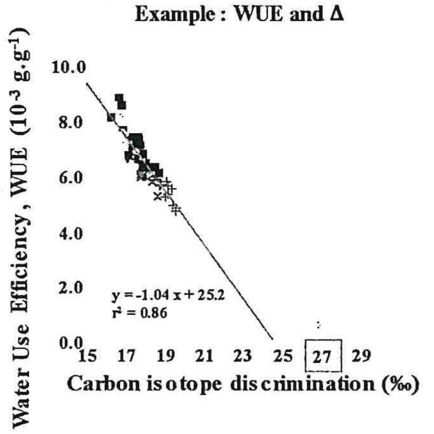
WUE = A/E
A = (g /1.6) * (Ca -Ci)
E = g (Wi - Wa)
WUE = [Ca(b- Δ)]*[1.6(Wi-Wa)*(b-a)]

- simultaneaous scale (A, E, g...)
- integrated scale (OM)

Background

Diap. 11

Example : WUE and Δ



Origines
Pinus pinaster
■ P. Vecchio
+ Tamjoute

example

Diap. 12

case between CO_2 and HCO_3- where the connection with C are higher in HCO_3-,: ^{13}C will concentrate in HCO_3- and α therm = 0.9910.

-Isotope discrimination (Δ): for practical reason, fractionation or discrimination is more used than isotopic effect.

$$\Delta = (\alpha - 1) \qquad (2)$$

When $\alpha < 1$, $\Delta < 0$ (negative) and $\alpha > 1$, $\Delta > 0$ (positive)

For example, for the transformation of CO_2 in HCO_3-, α = 0.991, discrimination will be equal to:

$$\Delta = (0.991 - 1) = -0.009 = -9.\ 10^{-3} = -9\ ‰$$

It means that during CO_2 hydration, the final product (HCO_3-) will be more rich in ^{13}C compared to CO_2 (it will be more rich 9 ‰). But during CO_2 dissolution, the CO_2 dissolved will see its proportion of ^{13}C decrease in a proportion around 1.1 ‰ compared to gas form.

DIAP 7

Other example: the isotopic effect during the carboxylation by Rubisco, measured *in vitro* will be

α = 1.0309 and the discrimination will be:

$$\Delta = (1.0309 - 1) = 0.0309 = 30.9.\ 10^{-3} = 30.9\ ‰$$

It means that if the quantity of substrate for Rubisco activity is not under limitation, the photosynthetic products will be more poor in ^{13}C compared to the subtract and this around 30.9 ‰.

This is true during *in vitro* measurement but not during *in vivo* measurement when there is a limitation of CO_2 coming by the stomatal aperture and the resistance of the chloroplast during diffusion phase.

Limited stage: when a chemical process is involving different successive steps (each steps will present a different isotope fractionation), the global fractionation of the total process will have the isotopic signature of the more constraints step.

For example, during photosynthetic assimilation of CO_2 in C_3 plants, the diffusion of CO_2 from the atmosphere to inside the leaf through stomatal conductance, the diffusion of CO_2 of the ambient air inside the leaf and the carboxylation by the Rubisco are two different steps which are discriminant (4.4 ‰ and 30 ‰ respectively) against heavy isotopes (^{13}C) towards the light form (^{12}C). If the stomata are open, the global discrimination is very near the carboxylation, but if stomata are closed (for example during a drought period), the discrimination will be more near of that from diffusion.

At the same time, during enzymatic reaction, which involve different steps in several sequences, if the limiting step is the activation of the enzyme or the substrat, or the complete formation of the complex enzyme-substract which is the thermodynamic stage (reversible) with a low fractional rate, the global fractionation will be low.

It is the case with the PEPcarboxylase. At the contrary, if the limited step is the final reaction, which is a non-reversible kinetic reaction, the isotopic fractional rate will be very high. It is the case of the fractionation during carboxylation by the Rubisco.

Isotope composition: there is a need to have international « etalon » to measure the isotope composition of different elements. It is the reason why an international standard has been determined as follow:

The ^{13}C isotope composition of one sample is defined by:

$$\delta\, ^{13}C = [(R\ \text{sample}/\ R\ \text{standard})-1]$$

DIAP 8

The international standard for carbon is a calcareous fossil, the Belemnite, which is coming from the Stone Age Pee Dee in South Carolina. It is named PDB.This fossil is rich in ^{13}C and the isotopic ratio is $^{13}C/^{12}C$ is equal to 0.0112372. In fact, the standard PDB doesn't exist but other standards have been calibrated with PDB before, will allow new calibrations now always in relation with it. The international standard for nitrogen is N_2 from atmosphere. For Oxygen and for Hydrogen, it is the ocean.

Most of the time, the organic samples and inorganic one are very poor in ^{13}C in relation with the standard PDB and $\delta\ ^{13}C < 0$.

Examples:

R of $^{13}C/^{12}C$ of ambient CO_2	0.0111473	$\delta\ ^{13}C\ CO_2$ air =-8 ‰
R of $^{13}C/^{12}C$ of C3 plants in average	0.0109338	$\delta\ ^{13}C$ C3 plant=-27 ‰
R of $^{13}C/^{12}C$ of C4 plants in average	0.0111136	$\delta\ ^{13}C$ C4 plant =-11 ‰

-Isotope discrimination

By combining the equation (1), (2) and (3) we can get:

$$\Delta = (\delta\ ^{13}C\text{source} - \delta\ ^{13}C\text{produit})/(1+\delta\ ^{13}C\text{produit})$$

($\delta\ ^{13}$Cproduit/1000) is negligible compared to one, we can write:

$$\Delta \equiv \delta\ ^{13}C\text{source} - \delta\ ^{13}C\text{produit}$$

Example:

Δ C3 plant (‰)$\equiv\delta\ ^{13}C$ (air) - $\delta\ ^{13}C$ (organic matter of C3 plant)

Δ C3 plant (‰)≡(-8 ‰)-(-27‰) ≡19 ‰

c. Methods and samples analyses

The principe of the measurement with the **isotope ratio mass spectrometer** (IRMS): the IRMS is measuring the isotope ratio ($^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$) of the pure gas (CO_2, N_2, O_2) and compare it to a reference gas (already calibrated with international standard).

The pure gas (sample or reference) is introduced in the « source » of the IRMS where it will be under a rain of electrons. The gas sample will be positively ionised. The gas is accelerated through focusing slits into flight tube where ion separated by a magnetic field through a 90° or 120°sector. They arrived in 3 different collectors in relation with their mass (44, 45 and 46 for CO_2; 28, 29 and 30 for N_2 and 32, 33 and 34 for O_2). Impacts are converted in Faraday cups into a voltage and subsequently into frequency. These signals are transmitted to a computer where inputs are analysed and mass isotopic ratio (45/44 and 46/44 for CO_2, 29/28 for N_2, 33/32 and 34/32 for O_2) are determined and compared to reference gas value.

Different systems in relation with IRMS will permit to analyse solid or liquid or gas sample with and without purification.

1. With basis analyser (analysis of CO_2 and N_2 of organic matter): the isotope ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) of one sample of organic matter is measured on CO_2 or molecular N_2 which collected under gas form the total carbon and nitrogen.

   This transformation solid-gas is done by a basic analyser in whom the combustion of the sample is obtained by mixing with oxygen in a helium atmosphere. All gas from this combustion is after separated in a gas chromatography column. The isotope analyse of the carbon will beard on 1 mg of organic matter (3 mg for the nitrogen) conditioned in dry fine powder.

2. Dual-inlet system (analysis of pure CO_2): the gas samples, collected during gas exchanges measurement as photosynthesis or respiration are purified on a « line » involved a vacuum pump and successive traps for water vapour and for the CO_2 . The purified CO_2 is collected in a vial and introduced in the « source » system of

the IRMS, and therefore analysed alternately with pure CO_2 from reference bottle. This method allows the automatic passage of 10 vial in the IRMS.

3-Triple traps (analysis of CO_2 not purified): the gas samples not purified are introduced in the IRMS but not directly to the source. These gases must be directed in a system of « triple traps » for purification before being introduced into the source. The « triple-traps » is in reality one « line » of purification, which is miniaturised and incorporated in the IRMS. A sample of 250 ml with 350 ppm CO_2 is enough for the measurement.

4. Loop system (analysis of CO_2 or O_2 in-line) : one loop (the volume of a loop is around 30 ml) installed before the column of gas chromatography of the elementary analyser and coupled with the system of gas exchange (open or closed) allow following « in-line » analysis : (i) the isotopic discrimination of the carbon during photosynthesis or (ii) the isotopic signature of the CO_2 respired from intact leaves. The sample of trapped air in the loop system go inside the gas chromatrogaphy system of the elementary analyser by the continuum flux of helium. The peak of CO_2 is individualised and oriented to the IRMS source

5 - GCIRMS (analysis of CO_2 or N_2 of liquid samples): it is an IRMS connected to a system of gas chromatography. A small quantity (?) of liquid extract send directly in the GCIRMS enter the gas chromatography column after vaporisation for sharing different metabolic peaks (sugar, organic acid, amine acid and aromatic composed). The metabolic peak (of interest) is after oriented to the oven and gas coming from the combustion go through the isotope mass spectrometer.

d. Possible applications of carbon isotopes

DIAP 9

1. Isotope discrimination against ^{13}C during the photosynthetic assimilation of CO_2 in the C_3 plants.

The plants in C_3 and C_4 make a discrimination against the ^{13}C during the photosynthetic assimilation of the CO_2. This discrimination is modelled taking into account the constraint stage. Farquhar et al. (1982) has elaborated one simple version for the C3 plants :

$$\Delta = a * [(pa-pi)/pa] + b * pi/pa$$
$$\Delta = a + (b - a) * (pi/pa)$$

This model presents the discrimination as a linear function of *pi/pa* (*pi* and *pa* are the partial pressure of CO_2 in the intercellular spaces and in the ambient air respectively). Only two main steps are taking into account during discrimination:

- the diffusion of CO_2 of the ambient air towards the inside leaf via the stomata (a)
- the carboxylation (b)

and he considered that there is no gradient of CO_2 between the intercellular spaces (*pi*) and the carboxylation sites in the chloroplastes (*pc*) with *pi=pc.*

The $^{13}CO_2$ presents a lower diffusion rate and a lower carboxylation rate than the $^{12}CO_2$, as a consequence the plant contains less ^{13}C than the CO_2 of the air. The discrimination during the diffusion stage throughout stomata (a) is around 4 ‰ and during the carboxylation (b) around 27 ‰. In fact, b is corresponding to the net « fractionation during carboxylation by two enzymes (Rubisco and PEPc) in the C3 plants:

$$B = b3\ (1-\beta) + * \ b4$$

b3 = isotope fractionation during carboxylation by the Rubisco (29 ‰) or (30 ‰, if the discrimination during dissolution of the CO_2 in liquid phase is taking into account)
b4 = isotope fractionation during carboxylation by the PEPc (-5.7 ‰ if the dissolution and the hydration of the CO_2 is taking into account), β = Fraction of carbon fixed by the PEPc in the C3 (β is around 5 %).

In the general standard conditions, the pi/pa =0.7 in the C3, the discrimination will be:

$$\Delta = 4.4 + (27-4.4) * 0.7 \equiv 20 ‰$$

It is possible to notice that the global isotope fractionation of the photosynthesis in the C3 plants is depending of constants (a and b) and of one physiological parameter *pi/pa*. The ratio *pi/pa* is under dependence of the relative importance of the diffusion towards carboxylation. The increase of *pi/pa* can be due to a bigger stomatal aperture or a less active carboxylase. **In these two cases the CO_2 fixation will not be the same.**

The species classification, genotypes and varieties related to the isotopic fractionation during photosynthesis is convenient to point out specific or genotype or differences, in limitation by stomatal and no stomatal origin.

The simple model was verified for numerous species C3 (i) at instantaneous step due to classical measurements of the variable *pi/pa* in the open system of leaf gas exchange and from Δ measurement by the analysis of the variation of δ 13C of the air (input and output) of the photosynthetic assimilation chamber (ii) at the day step by isotope analysis of the recent photosynthetats (soluble sugar) and (iii) at the total integrated step by the isotope analysis of the leaf dry organic matter.

e. Measurement of the internal leaf resistance to the CO_2 diffusion in the mesophylle in the C3 plants.

The simple version of the model described in following paragraph does not take into account:

- the mesophyll resistance to the CO_2 diffusion inside the leaf (until chloroplaste)

- the possible discrimination during mitochondrial respiration and photorespiration.

The simple model was validated for a lot of C_3 species. But for some grasses and trees, the "on line" isotope discrimination (Δo observed) was lower than expected (Δi, expected) by the model.

A complete version of the discrimination model taking into account all the process, which is corresponding to Δo was elaborated by Evans et al. 1986

$$\Delta o = a\ [(pa-pi)/pa] + s\ [(pi-pc)/pa] + b\ [pc/pa]\ -d$$

where s (= 1.8 ‰) represent the combined fractionation during the dissolution (bs = 1. 1 ‰) and the diffusion of CO_2 in liquid step (a = 0. 7‰ at 25 °C, O'Leary 1984)

d is dealing with the process of mitochondria respiration and photorespiration :
from the Fick 's law

$$A = (pi - pc) / ri$$
$$pi - pc = A * ri$$

A is the photosynthetic assimilation. If we look at the difference between the estimated value (Δi) and the observed one (Δo) =

$$\Delta i - \Delta o = ri\ (b - s)\ A / pa + d$$

We obtain a linear relation between (Δi - Δo) and A/pa with a slope which is proportional to the mesophyll resistance (ri) and at the Y-axis value at the origin (d) during respiration process.

The values of the mesophyll resistance calculated by this method are comparable with those obtain from leaf gas exchange and chlorophyll fluorescence. The values of d, very small, mean that the discrimination during respiration and photorespiration is negligible.

This model had helped to point out an important mesophill resistance for trees and some grass species more particularly those with thick leaves. In addition, it has been proved that the isotope signature of different genotypes of wheat more or less tolerant to the drought was correlated with the SLW (specific leaf weight) (Merah, 1999).

f-Isotope discrimination against ^{13}C during the photosynthetic assimilation of the CO_2 for the C_4 plants.

DIAP 9

The C_4 plants present a lower discrimination ratio that the C_3 plants. It is around 3-4 ‰ for the C_4 plant and 19-20 ‰ for the C_3.

The CO_2 is first hydrated in the cytoplasm solution of the mesophyll cell (equilibrium between CO_2 and CO_3H^-). During this reaction, the CO_3H^- is enriched in ^{13}C (around 9 ‰) relatively to the CO_2 . The PEPc is fixing the carbon under CO_3H^- on the PEP substrate to produce the AOA (acid oxaloacetic) which will do the malate. During the carboxylation, the PEPc will discriminate only around 2.2 ‰ at the contrary with the Rubisco, which discriminate around 29 ‰. The malate produced is moving towards cells of the perivascular sheath where it is decarboxylated and the CO_2 released is dissolved in the cells solution of the sheath and again carboxylated by the chloroplast of its cells by the Rubisco.

The carbon fixation under CO_3H^- , the very low fractionation rate by the PEPc and the sheath's waterproofs explain the very low photosynthetic discrimination of the C4 plants.

The simple model of photosynthetic discrimination in the C4 plants well described by Farquhar et al. (1982) and Deléens et al. (1983) is :

$$\Delta = a + (b4 + b3 * \Phi - a) * (pi/pa)$$
$$b4 = bs + bh + bp = 1.1 - 9 + 2.2 = -5.7$$

bs = fractionation during the dissolution of the CO_2 = 1.1 ‰
bh = fractionation during hydration of the CO_2 = -9 ‰ at 25 °C (negative because of the enriched CO_3H- in ^{13}C)
bp = fractionation during carboxylation by the PEPc = 2.2 ‰
Φ = rate of CO_2 release from the sheath to the mesophyll

The discrimination by the Rubisco in the C4 plant is related to the total CO_2 released from the sheath to the mesophyll

Example:

For a pi/pa=0.3 in the C4 plants, if $\Phi = 0$ (the sheath is totally waterproof: no discrimination by the Rubisco):

$$\Delta = 4.4 + (-5.7 + 30 * 0 - 4.4) * 0.3 = 1.3 ‰$$

If $\Phi = 1$ (the sheath is not waterproof: the Rubisco will discriminate as in C3)

$$\Delta = 4.4 + (-5.7 + 30 * 1 - 4.4) * 0.3 = 11.4 ‰$$

Between-tree variability of δ¹³C in maritime pine (*Pinus pinaster* Ait.)
Porté & Loustau 2001

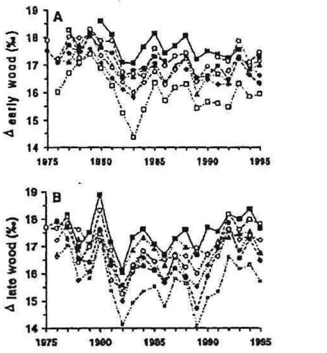
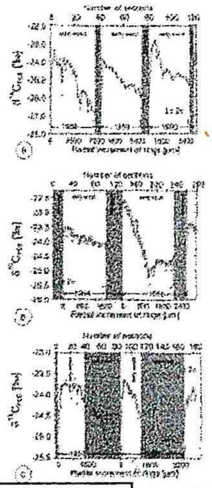


Figure 1. Interannual pattern of carbon isotope discrimination (Δ; ‰) measured on cellulose extracted from (A) early wood and (B) late wood of seven maritime pine trees. Each curve corresponds to one tree.

Diap.13

Intra-annual resolution of the δ¹³C signal in rings (seasonality signals).
Schleser et al. 1999

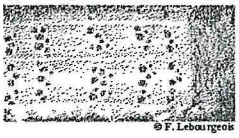


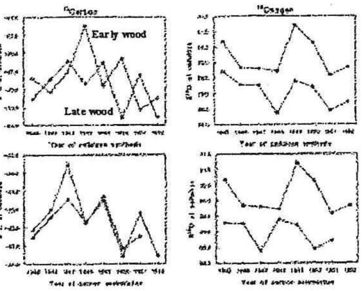
example

Diap. 14

Inter-annual resolution of the δ¹³C and δ¹⁸O signals in pedunculate oak.
Rapid recycling of triose phosphates in oak stem tissues. Hill et al. 1995

Quercus petraea ring porous species



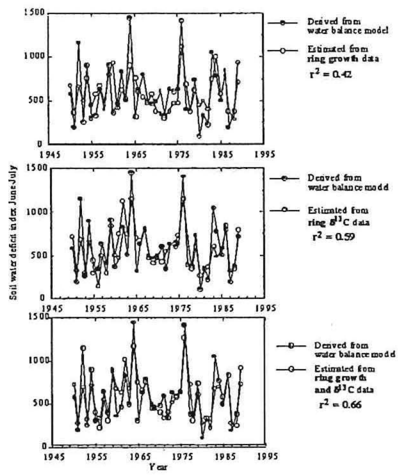


example

Diap. 15

➤ The inter-annual variations in δ¹³C are best correlated with soil water status in *Fagus silvatica*.
Reconstruction of soil water deficit using growth, δ¹³C (de-trended values) and growth + δ¹³C.
Dupouey el al. 1993

➤ Application to the assessment of site effects.

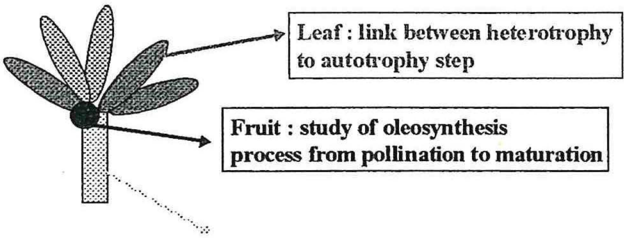


example

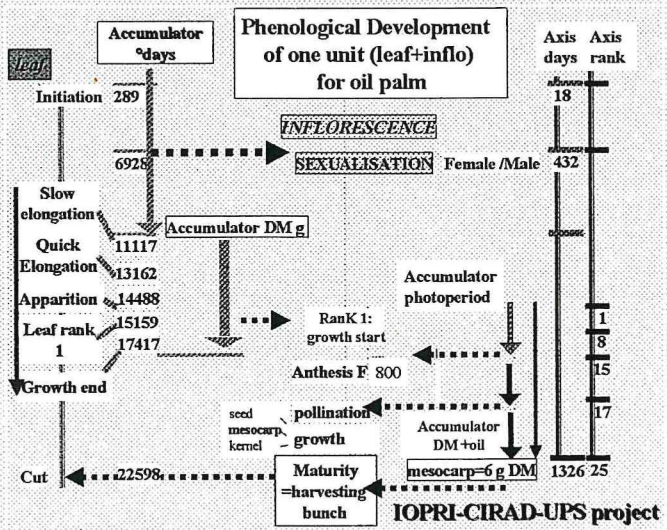
Diap. 16

IOPRI-CIRAD-UPS project

Carbon isotope discrimination :
« a method to investigate : (1) the transpiration yield of the dry matter production and (2) the use of the reserves in oil palm



Diap. 17



Diap. 18

This model of isotope discrimination has been validated for several C4 plants and it has been obvious that the structure of the different C4 plants (suberisation of the sheath and the position of the chloroplaste in the cells) was very much correlated to the isotope signature of the organic matter of theses plants.

g-Relation between the isotope discrimination and the water use efficiency in the C3 plant: indicator of the resistance to the drought in several genotypes.

The Water use efficiency (WUE) which is the "produced biomass: consumed water ratio" is an indicator of a plant's adaptation to drought. For C3 plants, the leaf processes that determine WUE are also those that control (delta ^{13}C (source) - delta ^{13}C (produced)), hence delta can be considered as a **way of directly evaluating WUE.**

DIAP 11, DIAP 12

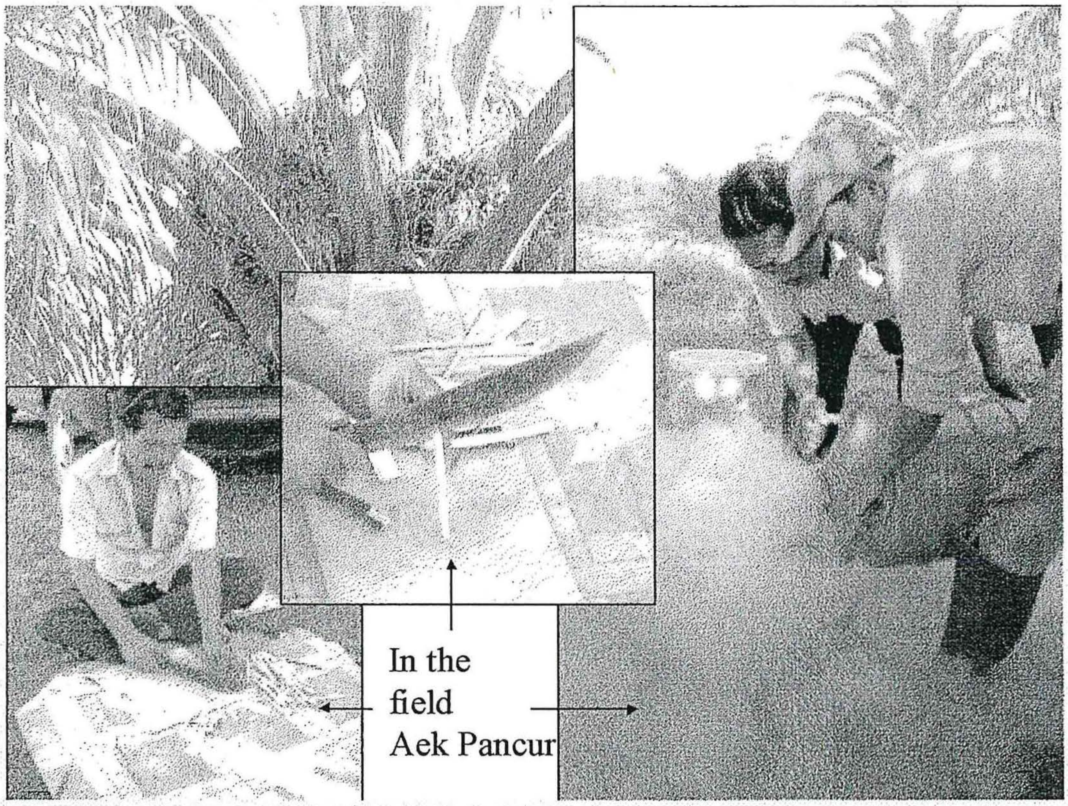
It has been shown, for a set of cultivated herbaceous species, along with forest trees, that WUE and delta are traits displaying substantial interspecific differences and high heritability. Hence, WUE and delta can be used as a selection criterion for breeding.

At the scale of a plant: WUE = A/E

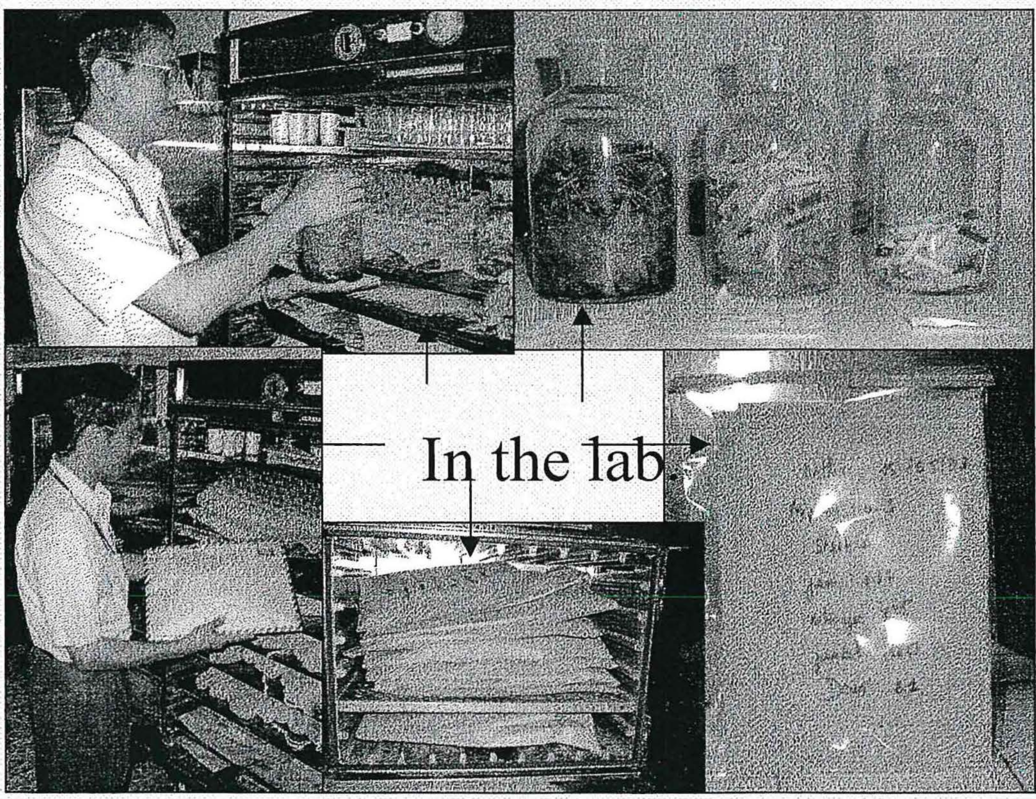
Where A and E are the CO_2 and the H_2O flux through the stomata. This flux could be described by:

$$A = (g/1.6)*(Ca-Ci)$$
$$E = g\ (Wi - Wa)$$

Where Ca-Ci and Wi-Wa are the differences of the concentration in CO_2 and H_2O between the ambient atmosphere and the intercellular space s and g is the stomatal conductance to the water vapour diffusion.



Diap 19



Diap 20

By combining previous equations, we can obtain the following equation:

$$WUE = A/E = [Ca\ (b - \Delta)] * [1.6\ (Wi - Wa)\ (b - a)]$$

This relation was proved by experiment at the instantaneous scale (in line measurement of discrimination), upon 2-3 days (isotope signature of soluble sugar) and at the integrated scale of development cycle (isotope signature of organic matter).

It may be obtained for different genotypes, different varieties or ecotypes, a negative linear regression between WUE and Δ.

In addition, given the difficulties encountered in measuring WUE in the field, a simple measurement of the carbon isotope signature of organic plant matter makes it possible to determine genotypes (varieties) that are more or less resistant to drought.

h. Does the isotope discrimination during dark respiration in C3 plant can be an indicator of the activity of the metabolic pathway?

The isotope discrimination during the photosynthetic assimilation of the CO_2 has been well studied and models applied to C3 and C4 have been developed and validated. But data in literature concerning the discrimination during the dark respiration are very few and contradictory.

Nevertheless, the organic matter integrates the plant functioning during light stage but also during darkness, consequently the isotope composition id related to both the photosynthesis and the respiration.

Every discrimination during dark respiration which released the enriched or impoverished in ^{13}C will modified the isotope composition $^{13}C/^{12}C$ of the standing organic matter related to the predicted values given by these models.

If a recent work (Lin and Ehleringer, 1997) shows that there is no isotope discrimination during dark respiration measured on isolated protoplastes of C3 and C4 plants incubated with sugar with an unknown isotope signature.

But maybe this discrimination still exists.

There is a heterogeneous distribution of ^{13}C inside the hexoses 's molecules pool and also it can be observed an isotope effect during the reaction catalysed by the pyruvate deshydrogenase (PDH).

The isotope signature of the CO_2 respired, compared to those of glucose, could be depending from the carbon fraction used in the Krebs cycle and the fraction derived towards the secondary metabolism (synthesis of terpenes, carotenoides, faty acid: lipids are poor in ^{13}C). The pyruvate is at one point of "branching", its relative contribution to the PDH reaction could modify the isotope effect of the PDH and then the isotope signature of the release of CO_2

From this pattern, it could be predicted that the isotope signature of the CO_2 released by respiration related to those of glucose might be different within species, environmental conditions and relative activities of the different metabolic pathways.

More recently, some results obtained in the laboratory UPS pointed out the existence of a substantial discrimination between ^{12}C and ^{13}C during the respiration of intact leaves (the CO_2 respired during darkness is enriched in ^{13}C relatively to the carbohydrates newly coming from the photosynthesis process see: Duranceau et al. 1999).

This discrimination is not constant: there is a variation between species (6 ‰ for the bean, 4 ‰ for the tobacco, 3 ‰ for the sunflower) and following the water status of the plant. During a drought period, the discrimination stays constant for the bean, increase for the tobacco and decrease for the sunflower (Ghashghaie et al. 2001)

Prospectives : new applications, elaboration of an INCO-DEV 6 Project for 2003 ?

Possible new investigations

-Differences between P/T/D in the field ,at nursery stage
-Identification of normal tissue culture materials
- Identification of drought tolerant breeding materials

INCO-DEV 6

Use of carbon isotope discrimination to explore genetic diversity and ecophysiological capabilities of perennial and tree crops

UPS : co-ordinator Prof. G. Cornic (NETCARB)
IOPRI, CIRAD, IRD, University of Melbourne (S. Arndt), IPB, ICRAF (?) + two european institutes, RUEDC (Dr Firman)

Diap 21

NETCARB

NETCARB is a Research Training Network funded by the European Community. It is based on cooperation of several ecophysiological laboratories on the use of stable isotopes for studies of ecosystem level and global carbon budgets.

This page is maintained by: badeck@pik-potsdam.de

List of publications by netcarb participants:

We select only publications related to our aims

Brugnoli E, Scartazza A, Lauteri M, Monteverdi MC, Máguas C (1998) Carbon isotope discrimination in structural and non-structural carbohydrates in relation to productivity and adaptation to unfavourable conditions. In Griffiths H (ed) Stable isotopes: Integration of biological, ecological and geochemical processes. BIOS, Oxford, 133-146

Duranceau M, Ghashghaie J, Badeck F, Deléens E & Cornic G. (1999) d ^{13}C of CO_2 respired in the dark in relation to d ^{13}C of leaf carbohydrates in *Phaseolus vulgaris* under progressive drought. *Plant Cell & Environment* **22**: 515-523

Gebbing T, H Schnyder, W Kühbauch (1998) Carbon mobilization in shoot parts and roots of wheat during grain filling: assessment by $^{13}C/^{12}C$ steady-state labelling, growth analysis and balance sheets of reserves. *Plant, Cell & Environment* **21**: 301-313.

Osório, J., Osório, M L, Chaves, M M, Pereira, J. S. (1998) Effects of water deficits on ^{13}C discrimination and transpiration efficiency of *Eucalyptus globulus* clones. *Australian Journal of Plant Physiology.* **25**: 645-653.

Picon-Cochard C, Nsourou-Obame A, Collet C, Guehl JM, Eerhi A (2001) Competition for water between walnut seedlings (*Juglans regia*) and rye grass (*Lolium perenne*) assessed by carbon isotope discrimination and d 18O enrichment. Tree Physiology 21:183-191

Scartazza A, Lauteri M, Guido MC, Brugnoli E (1998) Carbon isotope discrimination in leaf and stem sugars, water-use efficiency and mesophyll conductance during different developmental stages in rice subjected to drought. *Aust J Plant Physiol* **25**: 489-498

Diap 22

More emphasis could be done to check whether it could reasonably be assumed that isotope discrimination during respiration is an "indicator" of the metabolic pathways that are active in plants.

Isotope fractionation during biological processes and, consequently, the isotopic signature of organic matter and that of different metabolites depend on the availability (limitation) of substrate pools, of relative metabolite flows and of environmental conditions.

The isotope tool is a powerful tool for the analysis of mechanisms involved in the physiological functioning of plants, on different physiological scales (metabolism of cells, organs, individuals, ecosystem) and time scales.

Isotope fractionation models (in terms of their natural abundance) are now **"unavoidable" and complementary to plant physiological functioning and metabolic models.**

2. Application for oil palm

a- Objectives

The aim is to understand the phenology of oil palm by studying how the matter produced is distributed within the plants under different environmental conditions. A "probe", isotope discrimination is to be used to mark each phenological stage and understand the basis of development dynamics for two crops through precise monitoring of what happens to assimilates (growth, organogenesis, respiration, storage, and reutilization). This method provides a way of cold marking, over long periods, the photosynthesis products and respiration substrates that will then be used for development).

For oil palm, internal carbon management determines the existence and degree of sexual reproduction. Understanding the trophic mechanisms responsible for alternating male/female cycles in oil palm is one of the greatest challenges for agricultural research and oil palm breeding.

We wish to use an isotope discrimination "probe" in order to mark each phenological stage and **understand its sense by reconstituting carbon metabolic pathways** (induction due to water stress, or to the photoperiod, internal carbon balance between supply and demand).

Isotope discrimination is organic matter marking, and makes it possible to "track" carbon in an ecosystem or a living organism. **The ultimate aim is to produce a phenological model** making it possible to manage the crop through knowledge of its physiological functioning and control floral biology.

Already, a phenological comprehensive model **PHENOPALM** (Lamade et al. 2003) has been already elaborated following precise calendar established on LM2T x DA10D on Ivory conditions.

b- General context

1.oil palm phenological development

The oil palm is a **monoecious monocotyledon**, on which studies started at the beginning of the last century with the work byAdam (1910, quoted by Bredas and Scuvie, 1960). Throughout its development and growth, the oil palm produces alternating cycles of male and female inflorescences. The length of the cycles depends on the genotype and age of the oil palm, ecological conditions and the cultural techniques used (Jacquemard, 1995).

Moreover, the sex ratio is a very precise figure for each grower; it is the number of female inflorescence in relation to the total number of inflorescence for each palm

PHENOPALM

Parameters

NJSIMUL=5000	lenght of the simulation *(days)*
MSFMAX=3.6	DW max for a leaf (kg)
TEMPSEUIL=10	base temperature *(°C)*
DJINITEL=290	°days for initiation of a new pheno event
PERTOUR=10	harvesters ' rotation (days)
DURCROIFEUI=98	lenght of linear growth of a leaf
DJSEXUAL=6800	°days between initiation and sexualisation
SEUILAVORT=0.3	ITL min for the development of one inflorescence
DJCROIFEUI=10600	°days between initiation and growth starting
PCMSAPPAR=50	%of DM max during leaf apparition
PCMSRANG1=60	% of DM max during leaf rank 1
RGCROISINFL=11	rank of the begining of the growth of inflorescence
LATITUDE=4.5	latitude in °
SEUILPP=11.5	base photoperiod
CUMPPANTHMALE=240	cumulation of photoperiod for male anthesis
CUMPPANTHFEM=240	cumulation of photoperiod for female anthesis
RGREMPL=17	rank of begining of filling inflorescence
RGOLEO=25	rank of begining of oleosynthesis
MSMINOLEO=2	minimum weight for oleosynthesis
RGMATUR=30	rank of maturity of a bunch
RGELAG=35	rank of leaf prunning
OFFREFIXE=1.8	try (source = fixed)

Palm

Cumul Degre
N units

Unit

Cumul Degre
stage leaf
stage inflo
sexe
aborted
DM leaf
DM inflo
rank
CumPhotPériode
Date apparition
Date rank 1
Date cut
Date flowering
Date harvest

Different stages of a leaf

SF EDIFICATION=1
SF GROWTH=2
SF SPEAR=3
SF OPEN=4
SF ADULTE=5
SF CUT=6

Different stages of one inflorescence

SI INEXISTANT=1
SI INITIATED=2
SI GROWTHBANT=3
SI FLOW=4
SI FILLED=5
SI OLEOSYNTH=6
SI MATURE=7
SI CUT=8
FEMALE=1
MALE=2

LIVE one DAY
inspection HARVEST

Diap 1

Palm LIVE one DAY

***global demand** *(sum of all the units)*
***ITL** (supply/*global demande*)
***Accumulation of temperature**
(*process of initiation of new unit*)
***rank renumbering**
(life and renumbering of the ranks)
***harvesting** (the harvester coming every 10 days)

unit LIVE one DAY

*** demand** LEAF + **INFLORESCENCE**
(depending of the stage of development)
edification, cut **demand =0**
growth,spearleaf, full opening
***demand =DMmax/lenght of leaf growth**
adulte
- stage inflo
  - initiation, mature, cut **demand=0**
  - growth before ant. **demand=(0.833-0.017)/17.9**
    male **demand=(1.564-0.024)/17.9*(14-11)**
  - flow **demand=(1.231-0.833)/17.9**
  - filling **demand=(3.547-1.423)/17.9*(25-17)**
  - oleosynthesis **demand=(4.723-3.547)/17.9*(30-26)**

***accumulation °day** : initiation inflorescence °days
*** sexualisation** (ITL>1, if random <0.5*ITL then sexe=female, else male, threshold abortion =0.3)
* change stage
*** inspection harvesting** (if mature, cutting of bunch + leaf)

Diap 2

(females + males + early aborted). Up to now, the most frequent method used to predict yields in the next six months is to totals all the types of inflorescence for all the crowns of the sampled individuals. **A reliable prediction requires an understanding of the endogenous and exogenous mechanisms responsible for the cycles.**

To account for the alternation observed in inflorescence cycles (throughout the oil palm's natural range, both in potential zones for oil palm cultivation such as North Sumatra in Indonesia, and in zones with a significant annual water deficit as in the Ivory Coast and Benin), a set of hypotheses has been put forward regarding the effect of factors such as the water status of the palm (Adjahossou, 1983; Carvalho, 1991), the internal trophic balance (photosynthesis in relation to nutrients observed by Beirnart, 1935) or the involvement of hormones such as gibberellins (Huntley, 1995). Although numerous elements reveal a major genetic component in sex differentiation (Lamade et al., 1996), climatic events, such as periods with a marked water deficit, lead (statistically established on line LM2T x DA10D under the conditions at La Mé in the Ivory Coast by A. Flori) to the observation of aborted or male inflorescence 894 days after (period between sex differentiation and harvesting).

The same type of observation was closely carried out by Olivin (1966), Houssou (1985), Cornaire et al. (1993) in Benin. In addition, human intervention such as repeated castration-ablation causes total feminisation of the cycles, whereas severe pruning causes the appearance of male inflorescence.

It therefore seems that source-sink relations may be a prime factor in cycle alternations. As highlighted by Hartley (1988), it seems more likely that the amount of carbohydrates available has more of an effect on abortions than the sex ratio itself, which is more in relation to the genotype.

Recent developments in sex differentiation modelling (Flori and Lamade 2001 under way) and phenological rhythms (Lamade et al, 1998; Lamade, 1999) in the oil palm, based on daily variations in an internal trophic index (IT = C demand/C supply) depending on different phenophases (for fronds: stage 0: initiation to 289 °days; stage 1: slow elongation at 11117 °days; rapid elongation at 13162 °days; appearance at 14488 °days; opening at 15159 °days; cut during harvesting at 22598 °days. For inflorescence: sex differentiation at 6928 °days; growth at 15159 °days; Anthesis: photoperiod accumulator; Fruit-set: rank 17; harvest: rank 25 = 22598 °days) clearly produces, subject to further tests, an alternation in Female/Male/Abortion cycles.

The variations in the trophic index induced by climatic conditions on the one hand (drop in radiation, temperature, rainfall), and by specific physiological regulations in the oil palm (stomatal control, assimilate distribution, "storage or utilisation") lead in this elaborated model to variations in the organogenesis and growth rhythms that can have major repercussions on the abortion phenomena frequently seen on approximately 10 to 20% of fronds (Lamade et al., 1998), in a set of contrasting ecologies (Ivory Coast, Benin, Malaysia, Indonesia).

For the oil palm, abortion is possibly the visible direct adjustment to environmental conditions. **It is one of the cornerstones in mastering yield predictions for this plant.**

Field studies already undertaken by CIRAD in collaboration with La Mé, SRPH Pobè and Marihat Research Station:

Oil palm phenology:

-Phenological observations at the La Mé station (Ivory Coast) **from 1986 to 1992**, planting material: **LM2T x DA10D**. (produced from seed)

-Phenological observations at the Marihat station (Indonesia) **from 1993 to 1997**, planting material: **DA128 D self x LM7T self; BJ13D self x BJ221P** (produced from seed).

-Phenological observations, Pobè station (Benin) since 2000 by Leifi Nodichao: planting material?

-Phenological observations, PTP VII, Bekri, Lampung (South Sumatra, Indonesia) since 1997 **on MK93, MK54 and MK60** (clones). Need to be continued.

-Phenological observations in Tanjung Garbus and Aek Pancur (IOPRI, see further more)

2.Assimilate distribution

For oil palm, the first assimilate distribution model was developed by Dufrêne (1989), under the conditions at La Mé in the Ivory Coast. Assimilation of atmospheric carbon takes place via an initial photosynthesis module taking into account the maximum assimilation values for the plant (Ainf inf: the value of Amax when PAR is infinite), and the coefficient of light interception and apparent quantum yield.

The curve for photosynthetic response to radiation (PAR) is integrated in accordance with the cover (LAI) and in line with the exponential variation of radiation in the cover, model developed by Monsi and Saeki (1953). The carbon assimilated by a frond (source organ) is firstly used for growth requirements, then once those needs are covered, the remaining assimilates available go to "fill" the bunches in place. New developments in modelling are based on the internal trophic index (for each time step there is a ratio between the supply from the source and demand (growth and maintenance of standing biomass).

The main objective is to "indirectly" establish, through deductions from an annual carbon balance, a general functioning that is coherent with the yields observed in different environments. Based on observations carried out over the last 30 years by oil palm breeders, it clearly seems, for example, that bunch and fruit sizes, for a given genotype, of a given age, vary very little, with the main adjustment occurring either through retarded growth (net slowing down or halt to frond emission or opening), or through abortion (absence of inflorescence in the axil of some fronds: reduction in the size of the sink to be filled).

There is also alternation between development and organogenesis. For example, under the conditions in the Ivory Coast, the frond emission peak occurs (in May) after the main bunch harvest (in April), and therefore after major sinks has been removed. The nutritional condition of the palm may also be involved in promoting frond development, but few "functional" studies have been carried out on that level to date.

A model **SIMPALM** has been elaborated by CIRAD (Bonnot, 1995) on the base of the Dufrêne's model (Dufrêne, 1989) which gives an idea of the source supply for oil palm in different environment.

3- SIMPALM / PHENOPALM

The **SIMPALM model** is an interactive version of the Dufrêne's model written in C++. It is a simulation model about the oil palm yield. It is well described in Lamade and Setiyo (1996). A new version involving a water and nutrient balance model could be pertinent.

DIAP 1, DIAP 2

Albert Flori (CIRAD CP) Delphi has recently written the PHENOPALM model 5 environment (Lamade et al. 2003) totally based on phenological development on source/demand equilibrium, towards the daily elaboration of an internal trophic level.

It is preliminary model for the moment. It is giving on free simulated days (until 8000 days), male/female/abortion cycles for one tree. It also gives for each bunch, the date of anthesis and harvesting. For each leaves, the date of emission as well as the date of the full opening and final cut during pruning or harvesting.

This model is running at tree level: future development will lead to the simulation of a representative genetic population.

c- Materials and methods

1. Material

The clone MK60 (LM007T x DA 128 D) was chosen because it was already studied in Marihat Estate (Harahap, 1998) and in Bekri Estate (Lamade and Setiyo, 1997, 1998, Harahap et al., 1999) under *El Nino* effect and because it is also present throughout a multisite trial (cf. Lamade, Proposal 2002).

For the first preliminary step, we have decided to select in Aek Pancur, from 1995 planting, the following trees (MK60) , AP KLON 95 (D x P):

9/3 - 9/4 - 9/6 - 9/10 - 9/19 - 9/20 - 9/21 - 9/27

Before starting the isotope experiment, phenological recordings were done on these previous trees following the method (photo 1 and 2) already used in Andarasi (Lamade et al., 1998).

Others phenological recordings have been undertaken also in anjung Garbus, Afd III, PTP II, on the following trees (MK60) since February 02 with the prevision to study also "roots phenology":

14/1 - 14/2 - 14/4 - 15/4 - 15/3 - 15/1 - 16/1 - 13/1 - 13/2 - 13/3 - 13/4 - 16/4 - 17/17 - 17/18 - 18/19 - 16/18 - 15/20 - 13/17 - 19/17 - 15/19 - 14/19.

This method of following the leaf development has been used with success on previous phenological experiment (Dufrêne, 1989, Lamade et al, 1998): it may be considered as the best way to investigate the floral biology and the sexual development of oil palm.

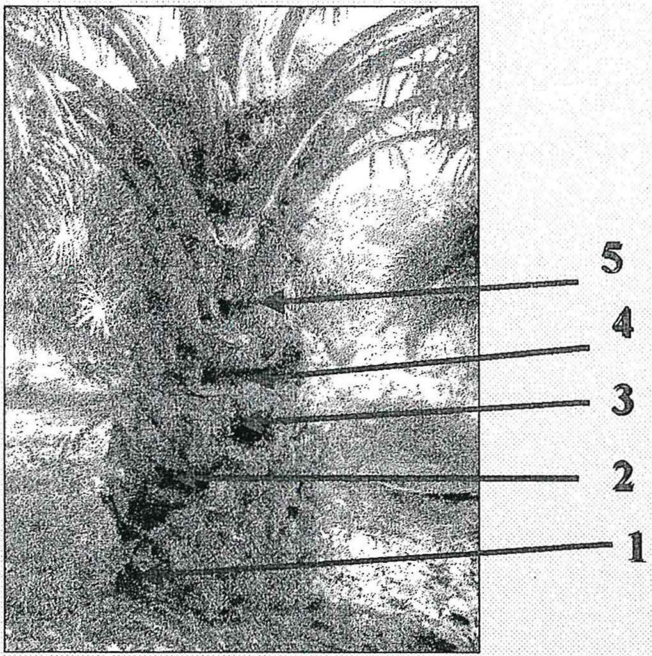
- Sampling method for carbon isotope analyses

We have followed the method done in UPS (Ghashghaie et al. 2001) and adapted it for oil palm. The minimum of dry matter collected must be up to 5 g: from that carbon isotope composition in DM, sugar or lipid could be easily done. But the sample must be representative of the chosen entity. It is the reason why we did collect fresh matter as much as possible (around 300 g to 1 kg of FM, depending on the organ)

After collecting samples on studied trees (from the trunk, the leaves or the fruits), they must be cut quickly in small piece (photo 7 and 9) due to the size of organs in oil palm, except for the fruits, before a very careful cleaning (but still softly) in ozone water (Photo 6) to avoid fungus infestation. Then, the sample is put in an icebox where frozen nitrogen is added (Photo 11). The samples must stay more than 10 minutes in frozen conditions to stop all metabolism processes.

Photo 13

5 locations on the stem for sampling OM



IOPRI-CIRAD-UPS project

1. Collecting OM in the field

2. Washing directly in ozona water

3. Put in frozen nitrogen >5 ', in the field

4. Put in oven <80°C

IOPRI

UPS

5. Grounding in fine powder in a ball mill

6. IRMS

Photo 14

All samples are put in a glass bottle and conditioned to be transported from the field to the laboratory . Double plastic bags and sticker are used to avoid any contamination.

Normally after nitrogen, samples must be put in a lyophilisator during 24 hours, but due to a lack of liability of the local one, they were put directly in oven as long as possible (DIAP 19 and 20) under a temperature < 80 °C and well conditioned until to be process in powder mill and analyse by mass spectrometer in IBP (Institut de Biotechnologie des Plantes) in Paris (France).

- Specific grinding process

For isotope analysis, samples must be very well grinded. In fact most of the samples were grindded almost three times. First of all samples of rachis and petiole as well as stipe were put in frozen nitrogen and grinded with a common blender (where it is possible to mix a big quantity of dry matter). Then aliquots where taken and grinded a second time with a smaller blender in addition with frozen nitrogen. Again new smaller aliquots were granddad in a precise and specific blender. The addition of frozen nitrogen at each step will permit to get a very fine powder.

d. Study of the stipe

- Objectives

As well as, previous studies done on European forest trees (DIAP 13,14 ,15) pointing out correlation between intra and inter-seasonal variation of $\delta^{13}C$, we wanted to investigate if the same phenomenon occurs for tropical crops as for oil palm among age and climatic conditions.

Because we still don't know anything about this variation, only five samples per tree studied will be collected for the first step. It is possible to see on photo 9, the five localisations on one oil palm where sampling was done.

- Specific sampling and process:

Isotope study : 8 trees are selected and phenologically recorded

Photo 2

Photo 1

Clone MK60, Aek Pancur, Medan
March 2003

Total sampling :

leaves : 8 leaves per trees (rank-2 to rank 5) x 3 = 120
per leaves : we measure the lenght AC

Photo 3

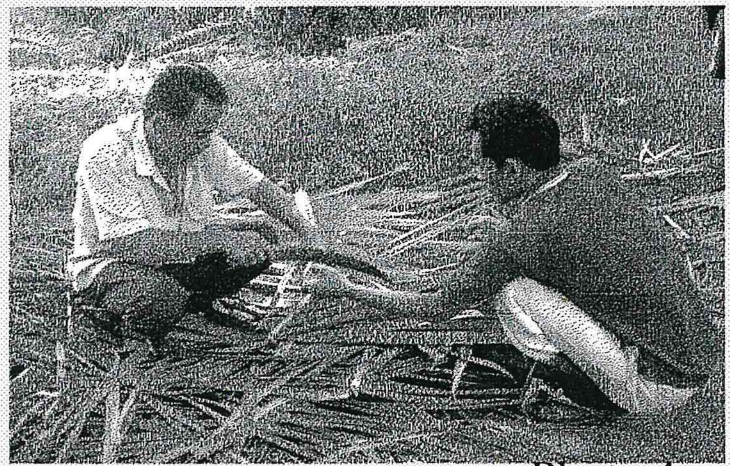


Photo 4

The leaf is divided in 10 equal samples, per sample : one piece of rachis, and two leaflets, as well as one piece of pétiole (minimum 50 g DM)

Only the live material has to be collected. After removing the dead part of the petiole basis, a volume equal to 30 cm x 30 cm x 4 cm is removed, cut in small pieces and directly entered, after cleaning in ozone water in a ice box full of frozen nitrogen during more than 20 minutes.

Then samples were put in an oven for more than 48 h under 80 °C, following the advice of UPS'team. Samples will be after send to France (UPS) for grounding to fine powder in a ball mill and weighed into tin capsules for isotope ratio mass spectrometry (IRMS).

One part of this powder will be keep to isolated starch in relation with the carbohydrate reserve pool.

- Post experimental treatment for destructive methodology:

To avoid fungus infestation after each sampling which provoqs a destruction of the stipe, pieces of absorbent cotton soaked with reagent were put on all studied trees. The main problem is also to avoid contamination of the new forthcoming organs. This last treatment must be handle with care.

e . Study of the leaf

It is possible to see, on the poster JEF, that 7 development stages can be noticed for the leaf organogenesis:

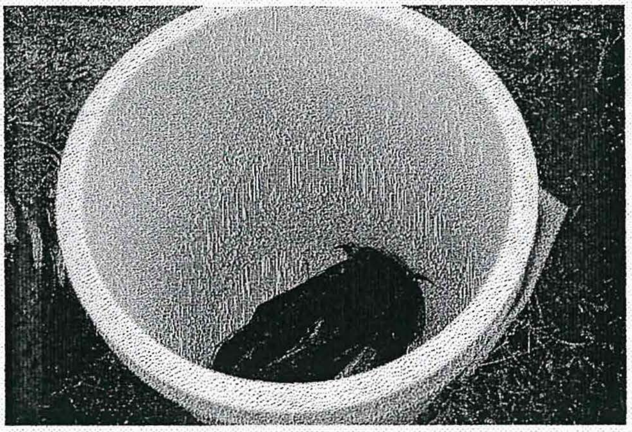
-**stage 1**: initiation that occurs every 18 days

-**stage 2**: slow growth from 666 days to 774 days if we take day 0 as an origin of the birth of one leaf

-**Stage 3**: quick growth from 774 days to 864 days

Measurement of the lenght of the rachis and the petiole
Aek Pancur, juin 2003

Photo 5



The leaflets are then put in ozona water and cleaned very carefully

Photo 6

The leaflets are then cut in very small piece and cleaned again

Photo 7

The same is done for the petiola

Photo 8

And the rachis...

Photo 9

-**Stage 4**: rank 0, corresponds to the emission of the leaf outside the crown that occurs at 864 days

-**Stage 5**: rank 1 that occurs at 882 after the "birth of the leaf",

-**Stage 6**: the end of the growth at rank 8 (1026 days)

-**Stage 7**: the pruning of the leaf during harvesting (1308 days)
(All numbers given previously are based on Ivory Cost observations)

As announced in the Proposal 2002, the first experiment is focusing from stage 3 to 5, which correspond to the leaf metabolism changes from heterotrophy to autotrophy.

- Specific sampling for leaves

Each leave, from the point C to A, is be divided in ten segments following the same methodology to determine the leaf area samples (photo 3, 4) of petiole and rachis are collected as well as two leaflets per segments (total 20). Leaves are always collected before 11 o'clock to avoid saturation phenomena and stomatal conductance closure. Collected leaflets are always put very quickly in frozen nitrogen.

They are previously cut in very small pieces and shortly cleaned in ozone water.

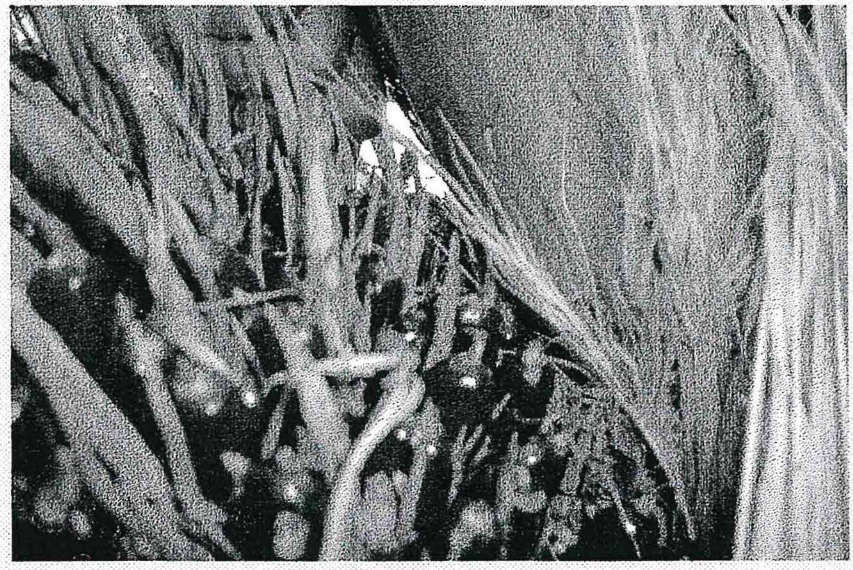
From leaves of rank 1 to rank 5, petiole, arches and leaflets are sampled separately. For leaves of rank 0, -1 and -2 ten samples mixing arches and leaflets are collected and cut in small piece.

- Problem of the drying of the leaves

The water content of the leaves is high and the drying in glass bottle provoke a water stagnation which could induce fermentation process. A crosscheck will be done by a new drying process.

Study of the fruits

For each growing stage, fruits are collected carefully



Bunch studied for clone MK60

Fruits at stage 3 after pollination

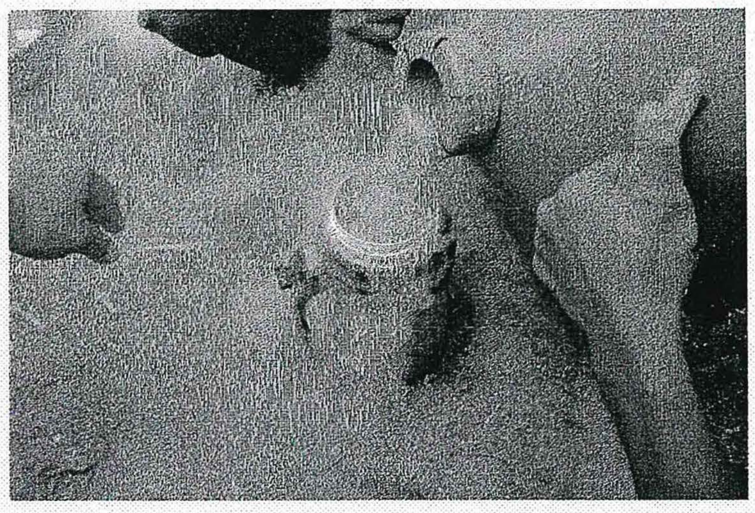
Aek Pancur, May 03

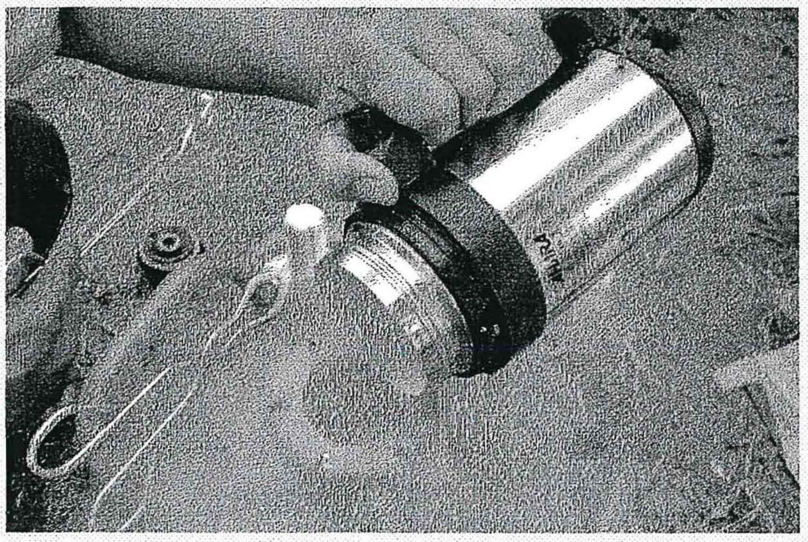
Cut samples are put in a ice box after being cleaned in ozona water

Photo 10

Liquid nitrogen is poured into the icebox. Samples stay around 20 minutes inside the nitrogen

Photo 11





After the recuperation of the samples nitrogen is poured again in the container

Photo 12

Aek Pancur, May 03

f. Study of the fruits

The general aim of this first study is follow the process of the oleosynthesis in oil palm fruits from pollination to maturation. For each oil palm tree the same inflorescence will be followed and sampling (photo) will be done almost every month until harvesting.

Just after pollination, fruits are very small, around one hundred will be needed to raise 30 g of DM.

3. Communication at the conference JEF

Poster presentation on oil palm phenology at the JEF2003 (Journées d'Ecologie Fonctionelle, INRA Nancy, France, 12-14 March 03

Investigating carbon demand for oil palm growth and development in different environments using phenological data"
Page 1, Page 2, Page 3

E. Lamade (1), I. E. Setiyo (2), G. Nizinski (3)

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Clear evidence has been brought recently the implication of internal trophic level in the seasonal reproductive trends of oil palm (*Elaeis guineensis* Jacq.) (Henson, 1999; Lamade et al. 2000, Noordwijk et al., 2001: WaNulCAS). Depending on biotic and abiotic

Investigating carbon demand for oil palm growth and development in different environments using phenological data

Emmanuelle Lamade (1), Indra Eko Setiyo (2) and Georges Nizinski (3)

(1)CIRAD CP Palmier, Montpellier, France;(2)IOPRI (Indonesian Oil Palm Research Institute) Indonesia; (3)IRD-UR Clifa-CIRAD AMIS Ecotrop, Montpellier France.

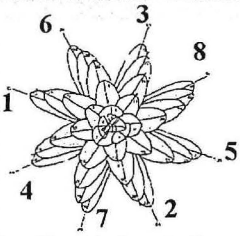
1. General phenological recordings from 4 to 6 years

Daily recordings

-date of each leaf apparition
-date of each full leaf opening
- sexe of inflorescence axilled by each leaf

-date of each inflorescence flowering
-date of each bunch harvested
-precise weight of the harvested bunch

Oil Palm Phenology



(8 spirals)

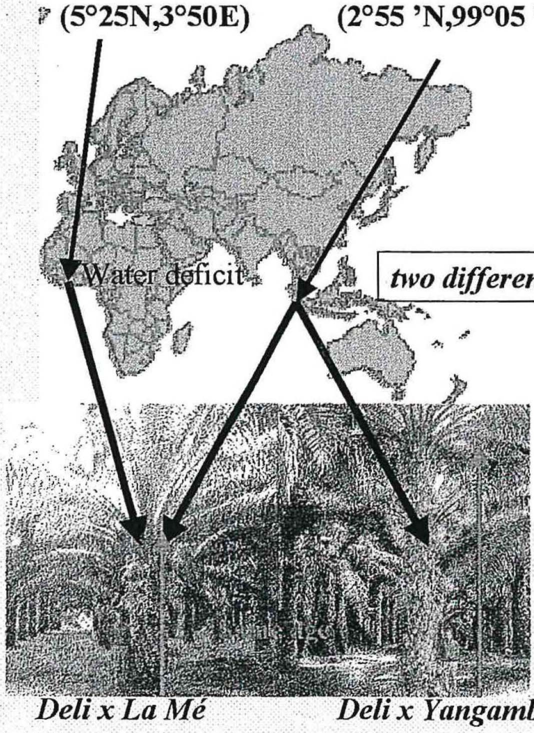
Specific « Left » phyllotaxy

Bottom-Up
Leaves quotation

2. In two different environments

Ivory Coast
La Mé Station
(5°25N,3°50E)

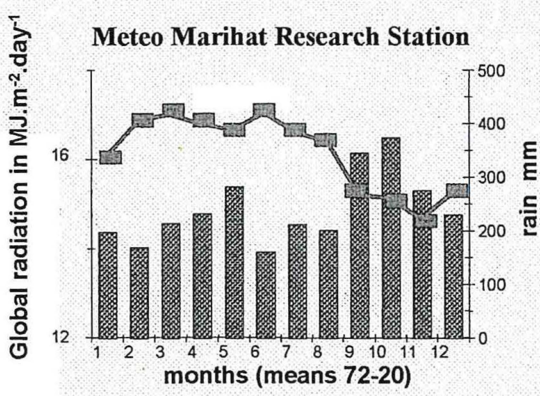
North Sumatra
Marihat Station
(2°55 'N,99°05 'E)

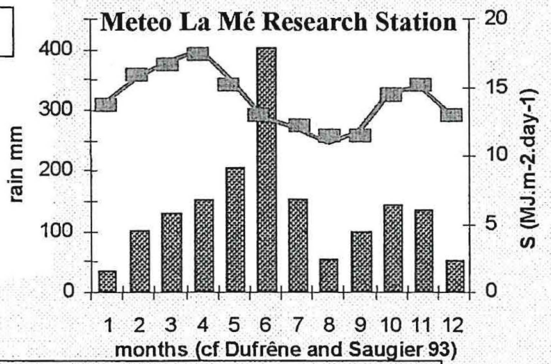


Deli x La Mé *Deli x Yangambi*

Photo from breeding oil palm programm with TDG himself

two different genotypes





Contrasting in :(cf Lamade et al.1998)
- *morphology and growth*
- *in sexualisation and production*
- *in phenological variations*

conditions, oil palm, a monocotyledon dioïc, produced alternatively male or female inflorescence in each leaf axis (Hartley, 1988).

The trophic state of each palm tree whatever environment is the result of the balance between sources supply and carbon demand. Until now, several simulation models (Kraalingen et al. 1989 :OPSIMM, Dufrêne, 1989 : NWRAYGBH, Harahap, 1998) have helped to a clear quantification, at the year scale, of the potential level of source supply in different environment and for different genotypes related to an evaluation of potential yield. Most of them as SIMPALM (Bonnot, 1994, Lamade et al. 1996) are based on ecophysiological characteristic as maximum photosynthetic rates, apparent quantum efficiency and canopy parameters as leaf area index (Dufrêne, 1989, Harahap, 1998).

Carbon demand in these model was often estimated from classical calculation cost (Penning de Vries et al., 1983) of annual increment of the standing biomass without distinguishing the competition between growing organs which may lead to different switches and priorities rules even in humid tropic environment. Very few have considered seasonal variations in the growth and the development of oil palm and as a consequence, a possible variation in the demand which, combined to the carbon supply rate, is susceptible to provoke a change in the internal trophic balance along the year.

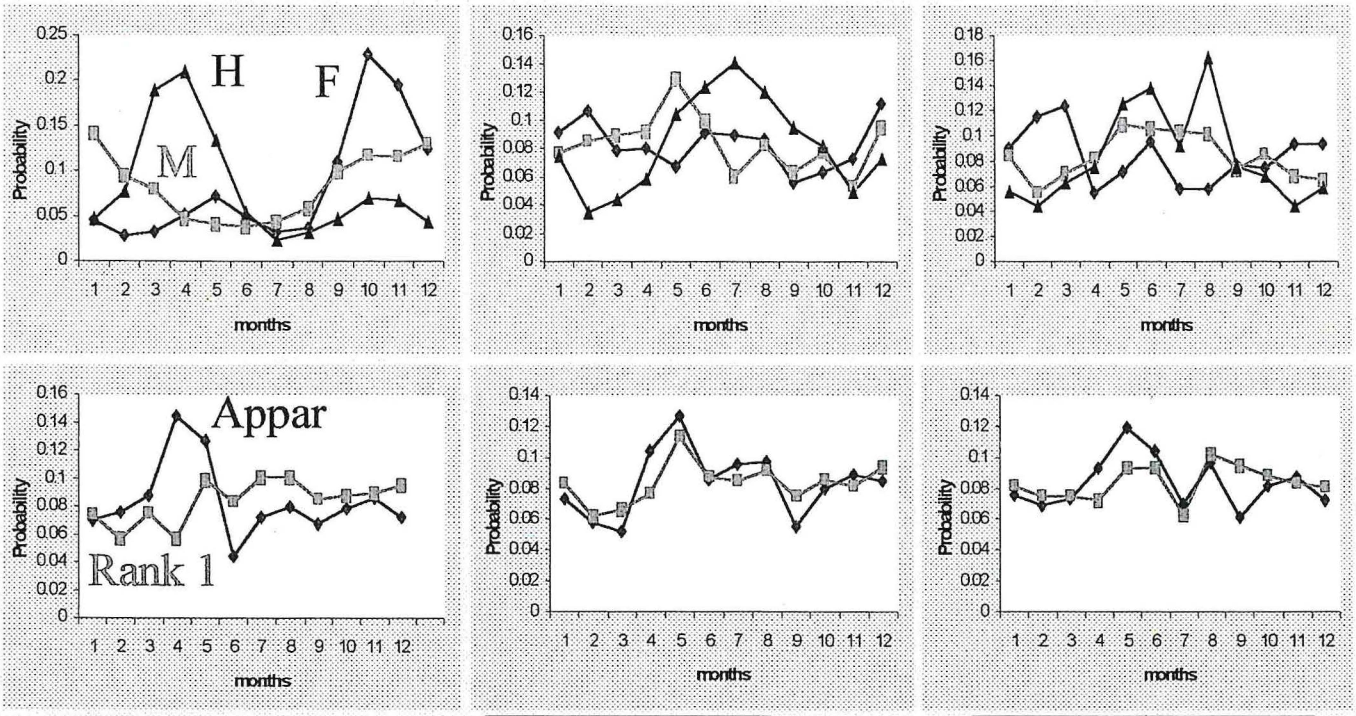
The annual variation of this trophic balance could be at the origin of specific switches and threshold governing organogenesis and oil palm sexualisation. On that base, a (stochastic) model predicting the sex of the new forthcoming inflorescence was elaborated from severe leaf and flower pruning experiment by Jones (1997).

It is the reason why, in a preliminary step to established a comprehensive development and phenological model for oil palm totally based on the seasonal evolution of an internal trophic index, we have investigated seasonnal carbon demand variation from precise information's obtained with daily phenological recordings, along a minimum of 4 years until 6 years.

Environmental and genetic comparisons will be also investigated throughout the study of two contrasting sites, one in North Sumatra (Indonesia) in potential conditions for this crop, the second in Ivory Cost which in dryer conditions, and two distinct families (*Deli x La Mé* and *Deli x Yangambi*). Elaboration of a new simulation model: PHENOPALM, totally based on phenological variation is the final objective of this present study.

Key words: *phenology, oil palm, carbon demand, internal trophic level, simulation model*

Example of seasonnal variations of phenology of oil palm in Indonesia and in Ivory Coast (Lamade et al. 1998)



Deli x La Mé Ivory Cost

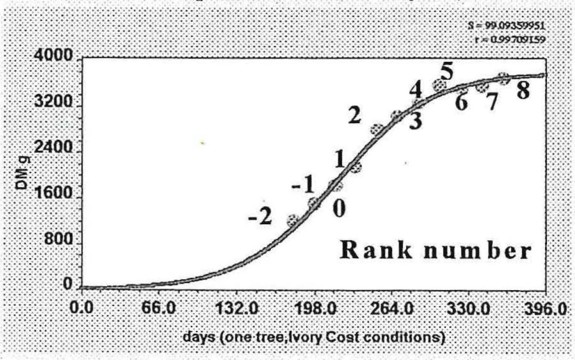
Deli x La Mé Indonesia

Deli x Yangambi Indonesia

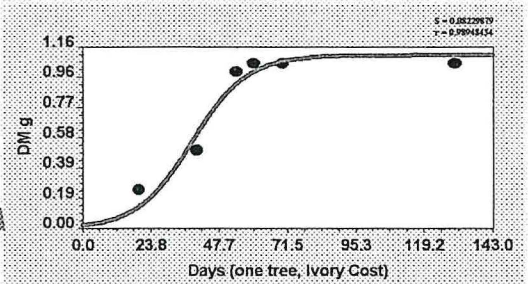
H: bunch harvesting, F: female flowering, M : male flowering, Appar : leaf emission, Rank1 : leaf rank 1

3. Example of growth trends per organs

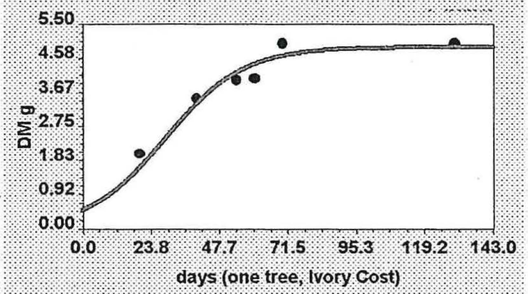
Leaf *(petiola, rachis, leaflets)*



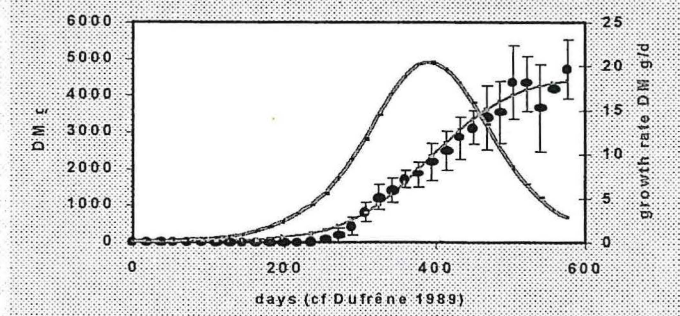
Kernel +oil



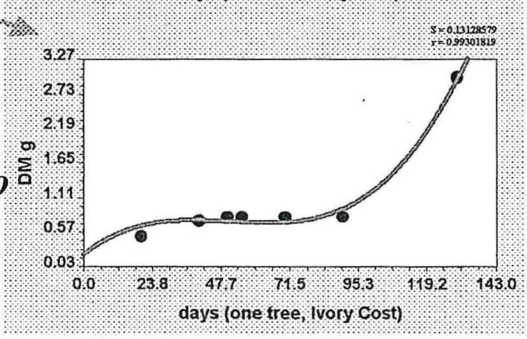
nut



Female inflorescence *(Ivory Coast condition)*



Meso carp +oil



Deli x La Mé

4. And carbon cost evaluation

leaf development							gCH20	g CH20
days	°days	stage of dev.	physio	stage DM	rank	biomass (g)	growth cost	maint. cost
18	288.5	initiation	heterotrophe	0	0	0		
666	11017.4	elongation	heterotrophe	0	0	5		
		slow						
774	13162.2	elongation	heterotrophe	5.7%weightmax	0	207		
		fast						
864	14844.5	apparition	heterotrophe	48%weightmax	0	1729		
882	15159.5	full opening	autotrophe	61%weightmax	rank 1	2220		
1026	17417.4	end growth	autotrophe	100%weightmax	rank 8	3625	5322	
1308	22335.4	cut	0	0	rank 24	0		1897
	weight	growth rate/day	growth cost	maint. Cost				
crown	g DM	g DM	g CH20day-1	g CH20 day-1				
with 47leaves	**128,425**	**201**	**297**	**2311**				

pheno stage	days	month	rank
leaf initiation	0	0	-48
sexualisation	414	14	-25
full opening	864	29	1
anthesis M	1139	38	14
anthesis F	1120		15
harvesting	1301	43	24

This is done for each palm studied

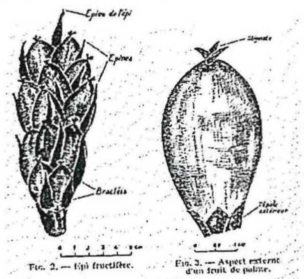


Fig. 2. — Épi fructifère. Fig. 3. — Aspect externe d'un fruit de palme.

						g CH20	g CH20
pheno stage		stages	sum pp	rank	biomass	growth cost	maint. Cost
sexualisation		phase0					
start growth		phase 1	326	6	0.04		
anthesis F		phase2	602	15	800		
pollinisation		phase3		17	1277		
oleosynthesis		phase4		25	9.4	12848	3520

	g CH2O	g CH2O
fruits	growth cost	maint. Cost
9.6 g FW	14.87	5.05
645 fruits	9591.15	3257
brakets+rachis	1479.88	263
(3.6 kg FW)		
DW/FW=0.28		
rang 25		

For one year, for 8 bunches, Deli x La Mé Ivory Coast , total carbon evaluation cost : 130.94 kg CH2O

5. *PHENOPALM* (elaborated by Albert Flori -CIRAD-Oil Palm Programm, in Delphi environment)

INPUT PARAMETERS

NJSIMUL=5000	*Lenght of the simulation (days)*
MSFMAX=3.6	*DW max for a leaf (kg)*
TEMPSEUIL=10	*base temperature °C*
DJINITEL=290	*°days for initiation of a new pheno event*
PERTOUR=10	*harvesters period (every 10 days)*
DURCROIFEUI=98	*Lenght of linear growth of a leaf*
DJSEXUAL=6800	*°days between initiation and sexualisation*
SEUILAVORT=0.3	*ITL min for the development of one inflorescence*
DJCROIFEUI=10600	*°days between initiation and begining of growth*
PCMSAPPAR=50	*% of DM max during leaf apparition*
PCSRANG1=60	*% of DM max during leaf rank 1*
RGCROISINFL=11	*rank of the begining of the growth of inflorescence*
LATITUDE=4.5	*latitude in °*
SEUILPP=11.5	*base photoperiod (hours)*
CUMPPANTHMALE=240	*Cumulation of photoperiod for male anthesis*
CUMPPANTHFEM=240	*Cumulation of photoperiod for female anthesis*
RGREMPL=17	*rank of begining of filling inflorescence*
RGOLEO=25	*rank of begining of oleosynthesis*
MSMINOLEO=2	*minimum weight for oleosynthesis*
RGMATUR=30	*rank of maturity of a bunch*
RGELAG=35	*rank of leaf prunning*
OFFREFIXE=1.8	*try*

1: number of new leaf
2 : day of leaf apparition
3 : day of leaf rank 1
4 : day of leaf cut
5: sexe of inflorescence
6: day of flowering
7: day of harvesting
8 : fresh weight of the bunch

OUTPUT

1	2	3	4	5	6	7	8
14	965	975	1690	F	1393	1660	42.4
15	983	993	1700	F	1408	1670	40.4
16	1001	1011	1710	M	1421		
17	1019	1029	1710	F	1436	1680	38.1
18	1037	1049	1720	M	1456		
19	1061	1075	1730	F	1485	1690	36.1
20	1090	1107	1740	M	1527		
21	1126	1149	1760	M	1585		
22	1155	1179	1780	M	1644		
23	1186	1215	1800	F	1684	1720	7.9
24	1216	1251	1820	F	1711	1730	5.7
25	1249	1286	1850	F	1728	1740	5.2

PHENOPALM is running at tree level :
it will be completed by the 44 palms studied in Marihat and tested on 22 palms in La Mé.

4- Communication for a seminar at IOPRI - May 03
Legend of the diapositives.

Diap 1: Tittle

Diap 2: stable isotope, general background

Diap 3: C3 and C4 plants, different stable carbon isotope composition

Diap 4: variation of isotope composition among individuals, organs, and molecules

Diap 5: fractionation or discrimination phenomena

Diap 6: definitions, isotope discrimination, isotope effect, isotope ratio.

Diap 7: Calvin cycles

Diap 8: isotope composition, international standard

Diap 9: examples of application of carbon isotope, the model of Farquhar et al. (1982)

Diap 10: model of Evans et al. (1986), correlation between SLW/Δ, relation between isotope discrimination and WUE.

Diap. 11: WUE (Water Use Efficiency), background

Diap 12: Pinus pinaster, example of relation between WUE and Δ

Diap 13: between-tree variability of δ 13 C in maritime pine (pinus pinaster)

Diap 14: intra-annual resolution of the δ 13 C signal in rings.

Diap 15 and 16: intra-annual resolution of the δ 13 C and δ 18 O signals in pedonculate oak

Diap 17: IOPRI-CIRAD-UPS project on carbon isotope

Diap 18: Phenological calendar for oil palm

Diap 21 : prospectives

Diap 22 : NETCARB references

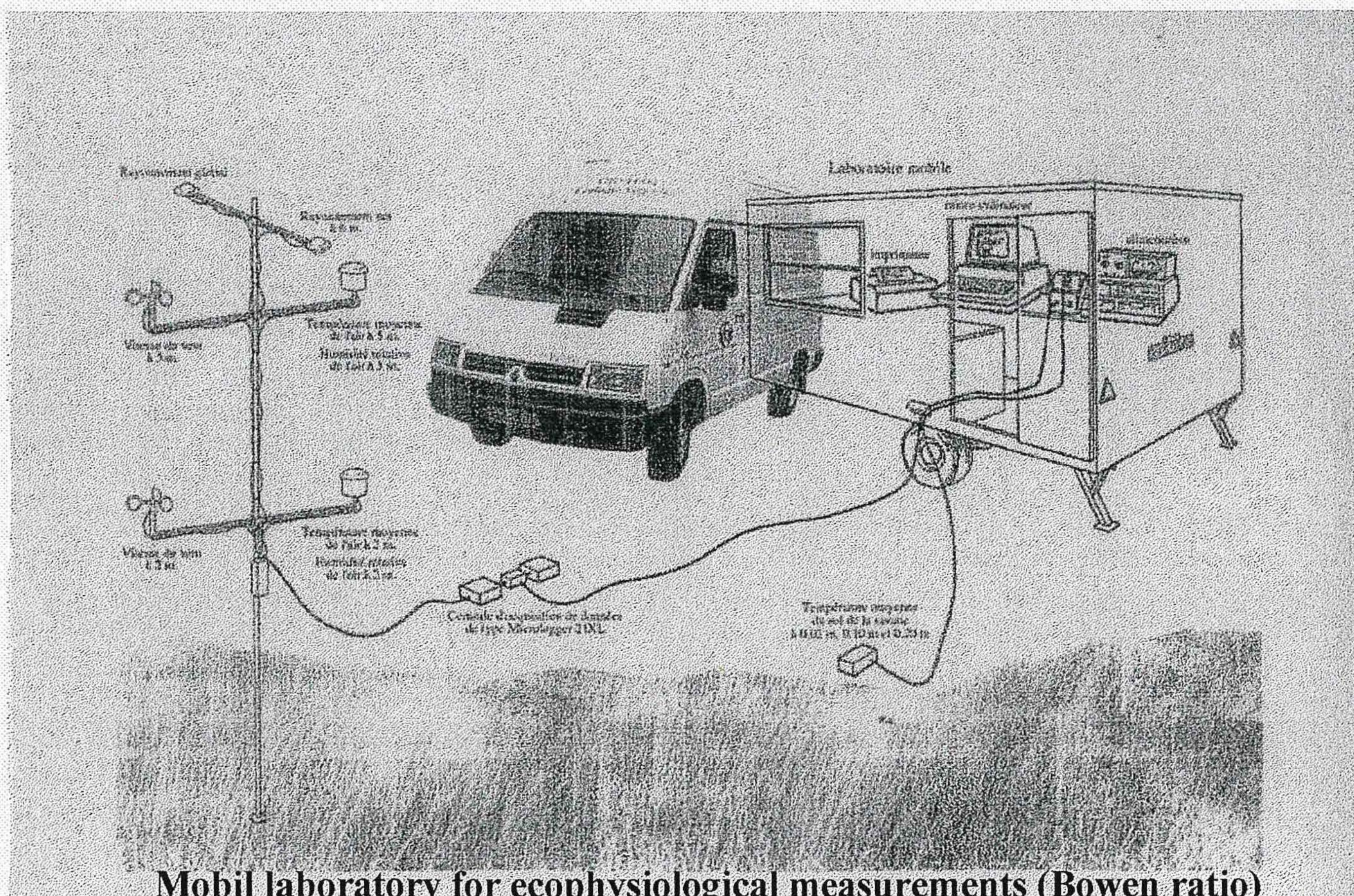
1 Sustainability of Eucalyptus plantations under drought conditions

2 Actual evapotranspiration and cover resistance of savannah

DIAP 1

Case study in Congo

Dr Georges Nizinski
IRD, France



Mobil laboratory for ecophysiological measurements (Bowen ratio)

DIAP 2

Part 2. Water balance: the method of Bowen ratio to estimate the evapotranspiration at plantation scale

1. Short report of Dr Georges Nizinski 's visit at Medan IOPRI (16-21 June 2003)

14 June 03: arrival of G. Nizinski at Medan

- Contact and discussion on a possible research programme proposed to IOPRI on the sustainability of forest ecosystem under drought conditions.
- Identification of a PhD D student on the use of Bowen ratio, first experimental planning on oil palm cover crop.
- Discussion on data process for phenological recordings following the phenological model elaborated by G. Nizinski in Journal of Applied Ecology.

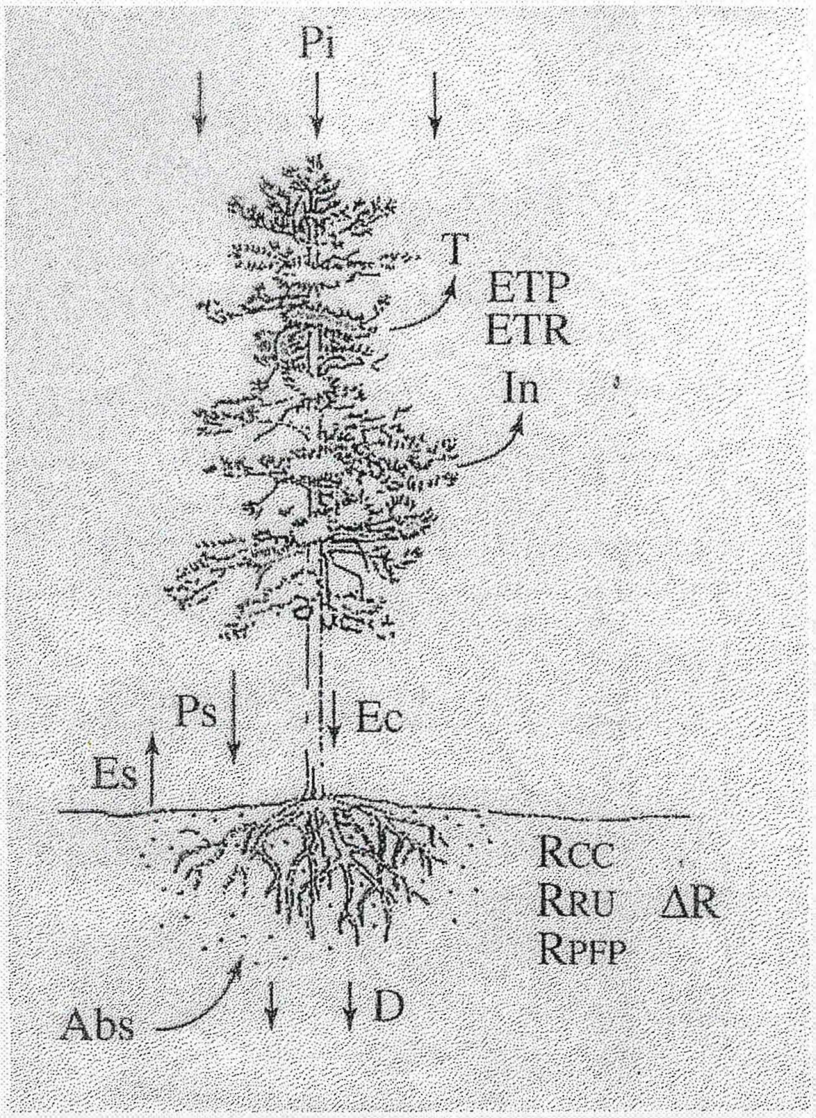
- Elaboration of the following manuscript for the journal "Sècheresse":
  Evapotranspiration réelle et résistance du couvert d'une savane à *Loudetia arundinacea* (bassin du Kouilou, Congo).
  Georges Nizinski, Emmanuelle Lamade, Joël Jean Loumeto, Joslin-Welcome Mouvondy et Edith-Crépin Founa-Toutou

16 June 03: meeting with Dr Darnoko and Dr Hasril Siregar (agroclimatologist, counter part CIRAD).

- Proposed project by Dr G. Nizinski with Dr Hasril Siregar and Emmanuelle Lamade on the comparison of 30 oil palm plantations from Aceh to Lampung involving water balance and phenology. Relation with the climatology following the results of the PhD D thesis of Hasril Siregar (Rancang bangun model penilaian

Schema of soil water balance under the plantation

DIAP 3



Evapotranspiration réelle de la savane - du 18 septembre au 11 octobre 1998

Date	Jour Julien	ETR du rapport de Bowen mm j⁻¹	ETP de Penman (48) mm j⁻¹	Rayonnement net (mesuré) cal.cm⁻² j⁻¹	Indice foliaire (mesuré)	Résistance stomatique modèle Jarvis-Stewart s.cm⁻¹	ETR de Penman-Monteith (65) mm j⁻¹	Retention en eau du sol (mesuré) %	Transpiration relative (Tr/ETP)	Interception net (mesuré) mm j⁻¹	ETR de l'équation du bilan mm j⁻¹
18-sept	261	3,14	3,81	276,34	3,30	3,6821	2,56	63,36	0,24	2,22	2,71
19-sept	262	2,16	3,41	194,85	3,35	5,4921	2,16	63,36	0,24		0,73
20-sept	263	1,54	3,14	80,60	3,39	15,1122	1,24	63,36	0,24		0,93
21-sept	264	1,87	3,98	163,53	3,43	6,7175	2,09	63,36	0,24		0,82
22-sept	265	1,86	3,85	161,49	3,47	6,8148	2,22	63,36	0,24		0,82
23-sept	266	1,55	2,11	144,55	3,51	7,7376	1,22	70,42	0,37	0,45	1,42
24-sept	267	3,32	4,38	290,48	3,56	3,4774	2,87	70,42	0,37		1,34
25-sept	268	1,42	1,86	128,48	3,60	6,8565	0,57	70,42	0,37		1,11

DIAP 4

kesesuaian dan dinamika iklim untuk perkebunan kelapa sawit, IPB Bogor, 2003). This will be developed in the next research proposal.

- Proposition of Dr G. Nizinski on the opportunity of training in different laboratories in France for Dr Darnoko, Dr Hasril Siregar

- Identification of **IOPRI's** candidate for a PhD D under the responsibility of Dr Hasril Siregar, Dr G. Nizinski et E. Lamade; Possible topics: methods to measure the effect of oil palm growth and the development on the variation of the depth of the water table. Comparison with a forest ecosystem and a grass ecosystem.

17 June (photo 1)

- Short visit of the experiment dealing with the CIRAD CP-UPS University of Paris XI, European project "NETCARB") and IOPRI PALM-ISO "Carbon isotopic discrimination: a method to investigate the transpiration yield and the role of reserve for oil palm". Sampling of the fruits at stage 3 on the clone MK60, plantation 95, (3 months after pollination) with the team ecophysiology-phenology of Marihat Research Station Marihat (Ir Simangunsum, Jack Marpaun, Polin Marbun)

18 June 03

Meeting with the agronomy department of IOPRI **Dr Darnoko et Dr Edy Sigit Sutarta** and Dr Hasril Siregar about following topics:

Dr Nizinski 's visit
at IOPRI , June 03

Photo 1

Aek Pancur, June 03

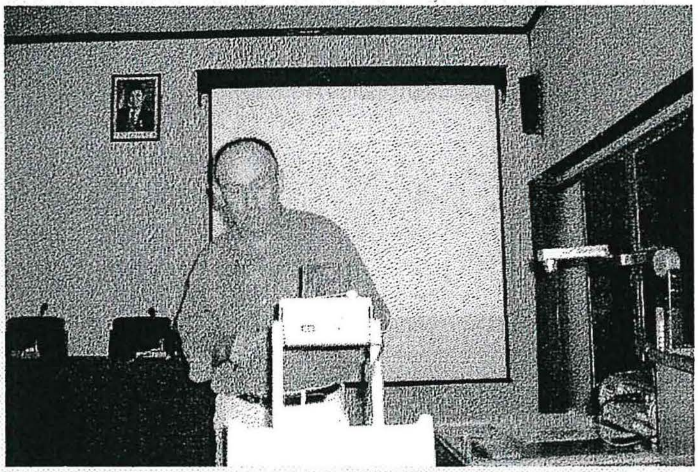
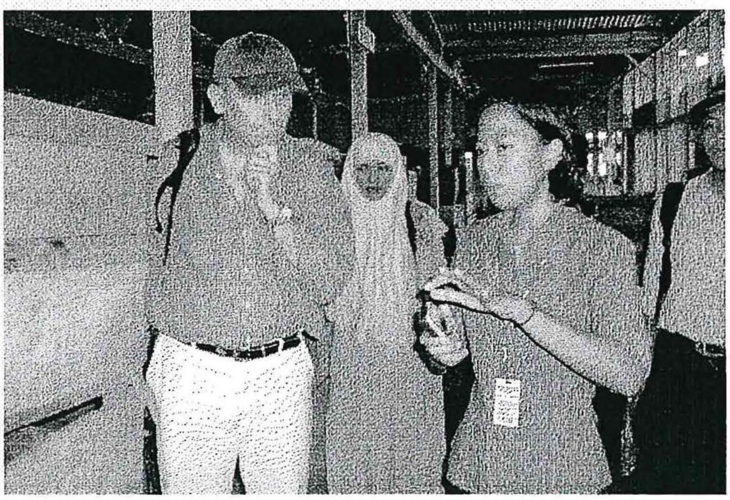


Photo 2

Aula Marihat , June 03

Marihat , June 03

Photo 3



Seeds Production Unit , Marihat
June 03

Photo 4

-Evaluation of water comsuption of one oil palm plantation
-Sustainability of oil palm under different ecologies following the spread of PTP plantation.

19 June 03- 21 June 03

Marihat Research Station (photo 2)

Presentation of Dr Georges Nizinski to the aula of MRS (Marihat Research Station, Pematang Siantar): "Sustainability of Eucalyptus plantation under drought condition". Co-ordinator **Dr Dwi Asmono**, scientific coordinator of Marihat (genetics and biotechnology)
Participants: staff of IOPRI, Marihat, RAPP (Riau). Questions on two tree crops that are more or less considered now as ecologically not friendly.

Possible elaboration of an ecophysiological project about selection criteria based on WUE (water use efficiency) connected with carbon isotope discrimination.

Discussion about the estimation of the evaprotranspiration and the resistance of the cover with the method of the Bowen ratio and its application to oil palm with **Dr Hasril Siregar.**
Visit of the seed production unit of Marihat with Miss Ir Yurna Yenni, Ir Firman Budiman and the present staff.

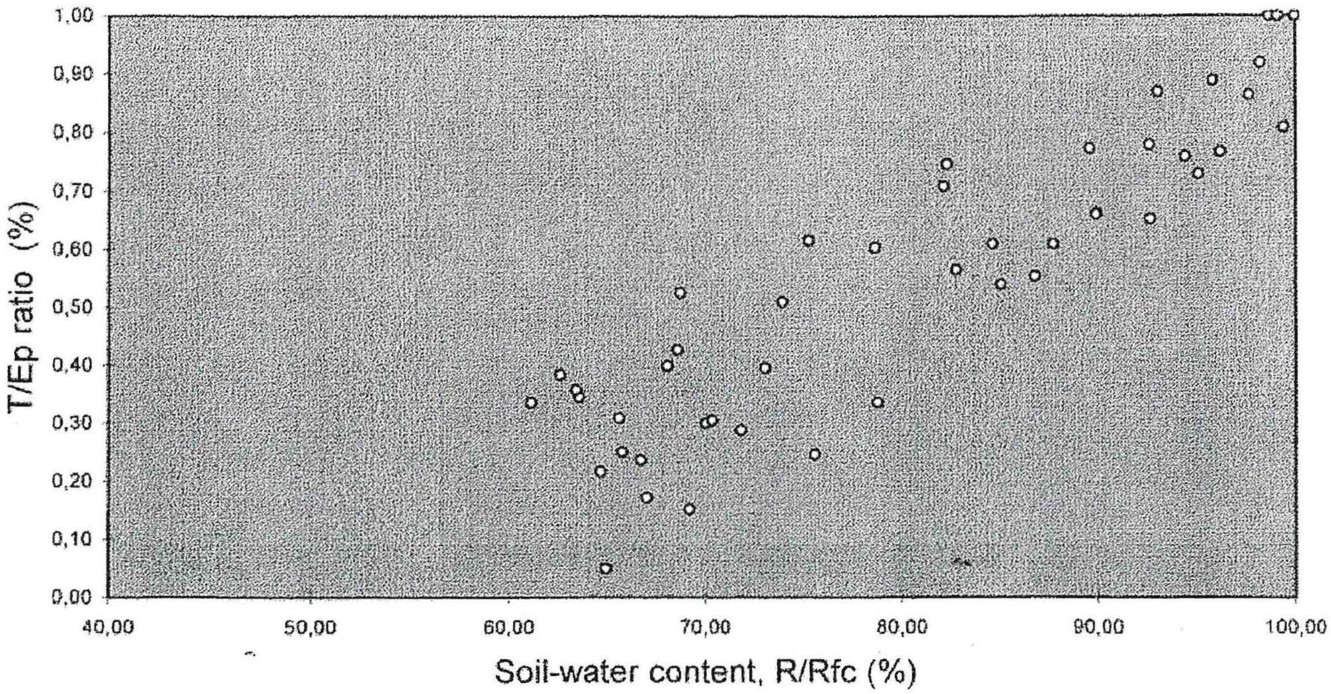
Visit of the tissue culture unit of Marihat with Ir Gale Ginting, director of Marihat; clonal material of oil palm, eucalyptus and banana trees. (photo 3 and 4)

2. Possible joint programme proposal in functional ecology for IOPRI-CIRAD-IRD period 2004-2007

From Emmanuelle Lamade and Georges Nizinski, Augustus 2003

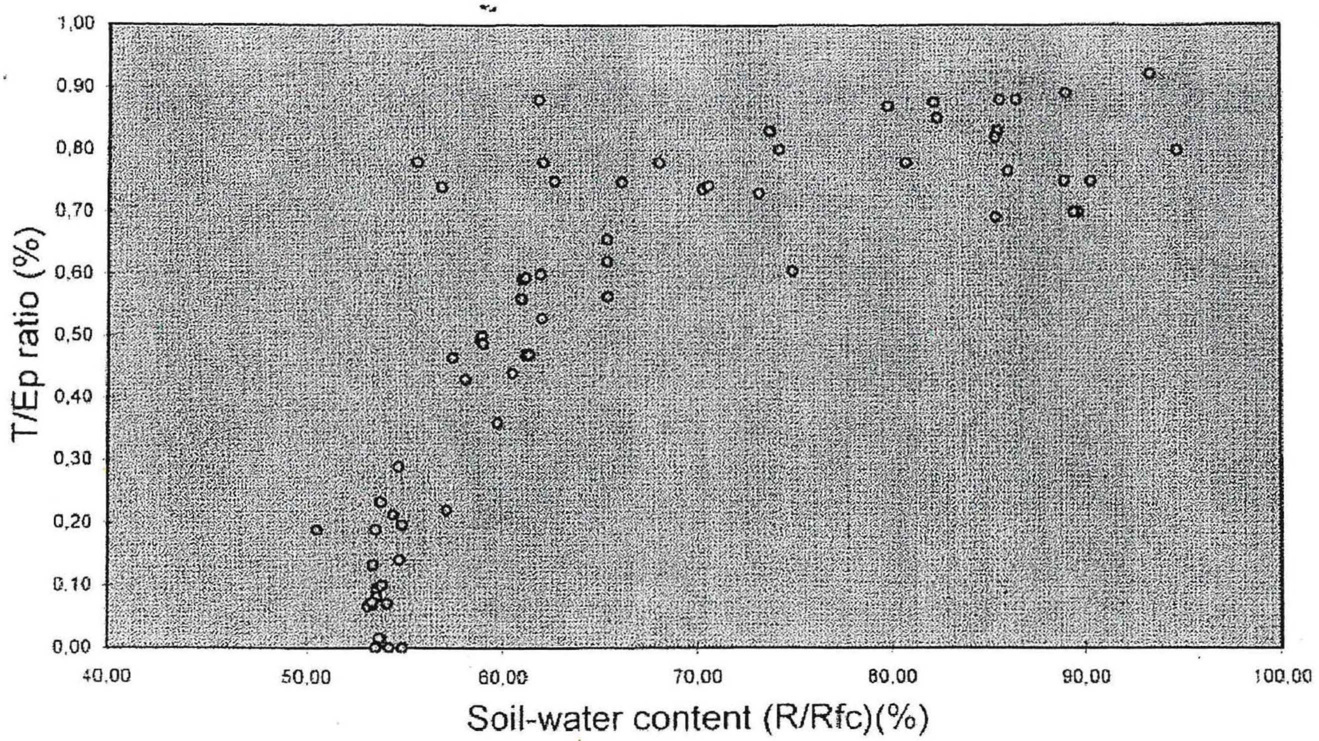
DIAP 5

Savannah



DIAP 6

Eucalyptus



"Influence of reforestation on bioclimatology and ecological functioning of perennial ecosystems along Sumatra Island (Indonesia): energy and mass exchange analysis of the system soil-vegetation-atmosphere - of two different tree crops, Oil Palm and Eucalyptus at plantation scale".

a- General background

The modifications of the terrestrial vegetation leads to change in radiation partition and latent heat flux /sensible heat flux at the soil surface which will influence the dynamic of the general circulation of the atmosphere, more specifically the rainfall which will provoke new evolution in the vegetation itself.

What will be the consequences of this reforestation on the ecological equilibrium and evolution of the Sumatra biome? What will be the sustainability of these agro- forestry-systems compared to the native ecosystems?

b- Possible Research programme 2004-2007

- Bioclimatology

Energy, water, carbon and nutrients balance must be compared within "native" and "planted" ecosystems (oil palm or eucalyptus plantation) at different stages on same environment to understand possible microclimatic changes due to the succession of different kind of vegetation.

Preliminary focuses:

-the adaptation of the Bowen ratio method to estimate the resistance of an oil palm plantation (or eucalyptus) at early stage.

-The comparison of two methods - the Bowen ratio and the Jarvis's model - to estimate the conductance of oil palm and eucalyptus population at different age and in different ecologies along Sumatra Island.

-The evaluation of the water table fluctuation under oil palm and eucalyptus in relation with the sustainability of each type of agroforestry system

- The adaptation of the Penman-Monteith 's formula to elaborate a new standard (compared to the classical IGM 12's method) for monthly water deficit evaluation for oil palm plantations along Sumatra Island gradient.

- Functional ecology

The understanding of the carbon cycle from the photosynthetic assimilation to the elaboration of the standing biomass and the quantification of the total CO_2 efflux are of key importance in the comparison of succession ecosystems.

In particular, must be emphasis:

- The evaluation of carbon stock and caption of species, oil palm and eucalyptus in potential conditions (North Sumatra). Comparison with local forest ecosystem

- the comparative carbon/nitrogen allocation priorities under water stress (water deficit and waterlogging) for two tropical species using carbon/nitrogen isotope discrimination (relation to the European project NETCARB) in different ecological conditions from the North (Aceh) to the South of Sumatra (Lampung)

- The adaptive patterns for phenology of both oil palm and eucalyptus related to global change in Indonesia

- Experimental planning

All experiments will be conducted by IOPRI in collaboration with the Indonesian network of the national PTP plantations from Aceh to Lampung.

Concerning oil palm (experiments will be first undertaken on this tree crop) Some sub-stations will be chosen in way to get a sufficient ecological gradient as far as radiation, temperature, rainfall and photoperiodism will be concerned along Sumatra Island. Attention will be paid on the genetic quality and representatively of the planting material studied (clonal material as MK60)

-in situ: sampling and collecting data (soil, vegetation, and climate) in different locations on several points (genetic material, age, and agroforestry practices).

-in controlled conditions, lysimeter

3. Expected output:

-Evaluation of the sustainability of the native ecosystem and the present agrosystem (oil palm plantation or eucalyptus).
- Quantification of energy and mass flux exchange between oil palm plantation (or eucalyptus and the atmosphere).

4. Example of a previous study in a savannah: elaboration of a new manuscript for the french review "sécheresse", 2003

"Measurement of savannah's actual evapotranspiration and surface resistance in the Kouilou basin, Congo"

Georges Nizinski[1], Emmanuelle Lamade[2], Joël Jean Loumeto[3], Joslin-Welcome Mouvondy[3] et Edith-Crépin Founa-Toutou[3]

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Summary

Measurement of savannah's actual evapotranspiration and surface resistance in the Kouilou basin, Congo

The aim of this work is to study the actual evapotranspiration and surface resistance of the savannah using the Bowen-ratio method for two contrasted periods, dry and rainy season.

The reliability of this method has been appreciated by comparison with the Penman-Monteith equation and the soil-water balance method in a 90 % *Ludetia arundinacea* dominated savannah (Pointe Noire, Congo: 4°34' S, 11°54' E; mean height 1.5 m; maximum biomass 3.5 t ha^{-1}; standing dead material 4.6 t ha^{-1}; mean annual leaf area index 5.3; rooting depth is 3 m; field capacity, R_{FC}=363 mm; permanent wilting point, R_{WP}=182 mm; available water content, R_{AW}=181 mm). Our results relate to the period from 18 September to 11 October 1998 (24 days): (a) from 18 to 29 September (« dry season »), the soil-water content was inferior than 70 % of the soil-water content at field capacity (63-70 % of R_{FC}; large soil-water stress; T/E_P from 0.24 to 0.37); (b) from 30 September to 11 October (« rainy season ») soil-water content close to 90-92 % of R_{FC}; no soil-water stress; T/E_P from 0.72 to 0.76). The mean daily surface resistance issued from Bowen-ratio method was 177.71 s m^{-1}, and 158.58 s m^{-1} during the « dry season » and 139.44 s m^{-1} during the « rainy season ». The total actual evapotranspiration (E_a) issued from Bowen-ratio method, Penman-Monteith equation and soil-water balance method were, respectively of 58.6-57.8 and 56.2 mm, with the mean daily E_a of 2.4-2.4 and 2.3 mm d^{-1}(2.4-1.5 and 2.2 mm d^{-1} in « dry season » and of 2.5-3.4 and 2.5 mm d^{-1} in « rainy season »).

The Bowen-ratio method was used for the calculation of actual evapotranspiration from the temperature and specific humidity differences, net radiation and the soil heat flux measurement: its advantages are a rapidity of installation, a temporal resolution of measurement (inferior to an hour) and a good integration of the heterogeneousness of the savannah's latent flux of vaporisation.

This method is adapted to the eco-physiological studies in the tropical conditions with reduced teams.

Keywords: surface resistance; Bowen-ratio; Penman-Monteith equation; evapotranspiration; soil-water balance

Références

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Legend of the diapositives

Diap 1 - Tittle

Diap 2 - Mobil laboratory for ecophysiological measurements (Bowen ratio)

Diap 3 - Schema of soil water balance under the plantation

Diap 4 - Actual evapotranspiration of the savannah in Congo from 18 September to 11 October 1988

Diap 5 - Relation between the soil-water content R/Rfc (%) and the ratio T/Ep ratio (%) for savannah ecosystem

Diap 6 - The same than previous but for Eucalyptus plantation.

Part 3. Agronomy : example of INCO projects for the following years.

1. INCO project from Dr Frank Enjalric :AGITAF project

"Agro-environmental Indicators for Tropical Agro-forestry cropping systems adapted to sustainable rural development"

Co-ordinator : Dr. Frank Enjalric, CIRAD, France

This project will be proposed next year to the European commission if that kind of topic is still receivable.

Summary

Perennial crops have a life expectancy between 35 years (rubber) and 70 years (coconut), including a growth phase without production often called «immature period». Farmers usually take advantage of this period with inter cropping. A large variety of inter-cropping schemes can be observed with various levels of intensity and efficiency. This valorisation is a key to sustainable rural development.

A better understanding of cropping systems performance and their agronomic functioning would permit to widen the choice of agricultural techniques and to find ways to improve the farming systems.

This project will focus on multi-species cultivated plots, where perennial crops and annual crops are intimately mixed.

The main objective is to identify biophysical indicators to estimate performances of some crop associations and agricultural practices that are able to alleviate farmer's constraints and to solve problems on farming systems sustainability, especially in term of long term fertility management.

The functioning of cropping systems on particular plots will be analysed in order to:

i) Identify and assess some agronomic indicators able to evaluate and to monitor the behaviour and performance of cropping systems in time,

ii) Provide simple tools for *in situ* monitoring of plots,

iii) Improve performance of these cropping systems by solving problems of the respective sectors

iv) and lastly to permit simulations for adjustment and/or conception of new systems

<u>Documentation:</u>

Izac, A-M. N., 2003. Economic aspects of soil fertility management and agroforestry practises. In Trees, crops and soil fertility, Ed. G. Schroth and F.L. Sinclair. Cabi publishing.437 p.

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Lal, R. A., 1989, Agroforestry systems and soil surface management of a tropical alfisol. Agroforestry systems 8: 1-6

Schroth, G., 1995, Tree root characteristics as criteria for species selection and systems design in agroforestry. Agroforestry systems 30: 125-145.

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2 Inco's project : ITEM-Palmoil

Co-ordinator : TTZ Bremerhaven - Environmental Institute

Integrated **Technology**- and **Management** approach for a sustainable **Palm oil** production in southeast Asia

INCO-2002-A2.1

Key word : oil palm, palm oil, sustainable production, POME treatment, composting, integrated knowledge based system, biodiversity, land rehabilitation, Asia.

SUBIMTTED THIS YEAR, INCLUDING IOPRI AND CIRAD

Due to the rising demand worldwide, the world palm oil consumption is expected to grow further (the growth rate is estimated in 3,5% per year). This production rise will be increasing the pressure on untouched rainforest areas in South-East-Asia. From the production point of view, the cultivation of rainforest areas would allow to match the increasing demand but would destroy valuable ecosystems and biodiversity in the main producing countries.

However, the clearance of high conservation value forests, the extremely polluting residues generated during palm oil production and the nutrients consumptive monocultures pose serious problems to the regional environment and the local communities. Indeed, faced with increasingly restrictive environmental legislations and the need to be competitive against other producing countries (for instance in South America) many small and medium producers and farmers are forced to look for innovative and sustainable alternatives.

In order to protect the biodiversity and to use the local resources (soil, climate, vegetation) in a more sustainable and efficient way the establishment of an integrated production strategy for the production of palm oil is necessary. The here proposed strategy includes several key aspects like the appointment of an indicator system for sustainable palm oil production, an advanced soil and water management, and wastewater treatment with closed loops, the increase of production per hectare through the use of mycorizza (field tests have shown an production increase of 68% per hectar), and a reuse of biological waste through composting.

Against this situation, the proposing partners (Palm oil producers, Research Institutions, Environmental companies) intend to take an integrated and more proactive approach to the problem: the aim is to develop an integrated and sustainable strategy for the palm oil production in South East Asia. This concept will integrate social, ecological and economic aspects and criteria's of sustainability. The main focus will be on the following modules.

The main focus will be on the following modules.

1. Assessment and characterisation **"status quo"** of current facets and interactions for palm oil production in regard to social-, ecological-, economical-, legal- and technology aspects

2. Establishment of a **"round table"** of stakeholders (farmers, oil producers, local government, environmental organisations etc.)

3. **Development and implementation of management practices** for sustainable palm oil production to be established and demonstrated within the project

   Verification criteria: Number of sustainable management strategies developed, obtained improvements in relation to social, ecological, legal and technological aspects

4. **Development and Implementation of the IKBS** as complementary tool together with the demonstrated practices to function as knowledge database, decision support and information platform appropriate to different actors

   Verification criteria: Knowledge bases containing the expertise of different actors (mill owners, plantation owners, worker, local actors) on each topic, analysis of knowledge flows amongst actors and analysis of knowledge gaps

5. **Development and Implementation of techniques and technologies in a real palm plantation:**

   - **use of mycorrhizal fungi**
   - **restoration of natural forest ecosystems / biodiversity conservation**

   protection concept for neighbouring ecosystems

   composting system advanced water management system

Crop yield
in interaction

Long terme effect
on fertilisation

Competition
for water and
nutrient

$$YC = Y0 + F1 + F\omega + C_l + C_{W+N} + M$$

yield
in monoculture

Direct Effet
of the fertilisation

Competition
for the light

Effet
of
microclimat

1. Experimental level

Residual effect

Roots barrier

Transfer (mulch)

Removing trees

2. Level of fonctionnal knowledge SOM

roots architecture

Liter Quality
level of mineralisation

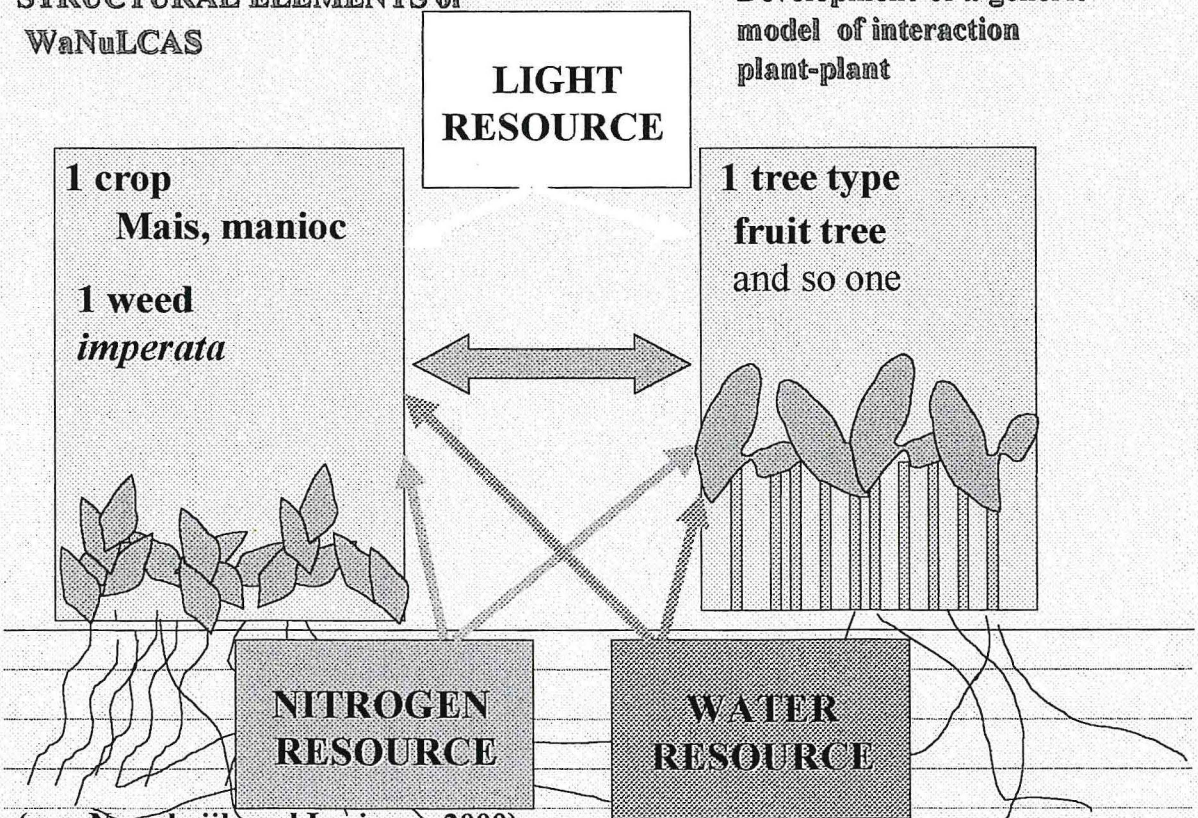
Light profil

3. Level of synthesis WANULCAS

(van Noordwijk and Lusiana, 2000)

STRUCTURAL ELEMENTS of WaNuLCAS

Development of a generic model of interaction plant-plant



(van Noordwijk and Lusiana , 2000)

6. **Evaluation of implemented techniques, technology and management practices as well as the IKBS**

   Verification criteria: user's feedback,

Part 4. Presentation of the WaNuLCAS platform (page 1 to 7)

1. Introduction

WaNuLCAS 2.1, a model of Water, Nutrient and Light Capture in Agroforestry Systems has been elaborated by Meine van Noordwijk (Van Noordwijk, M. and Lusiana B. 1999) at ICRAF Bogor (International Centre for Research In Agroforestry) .

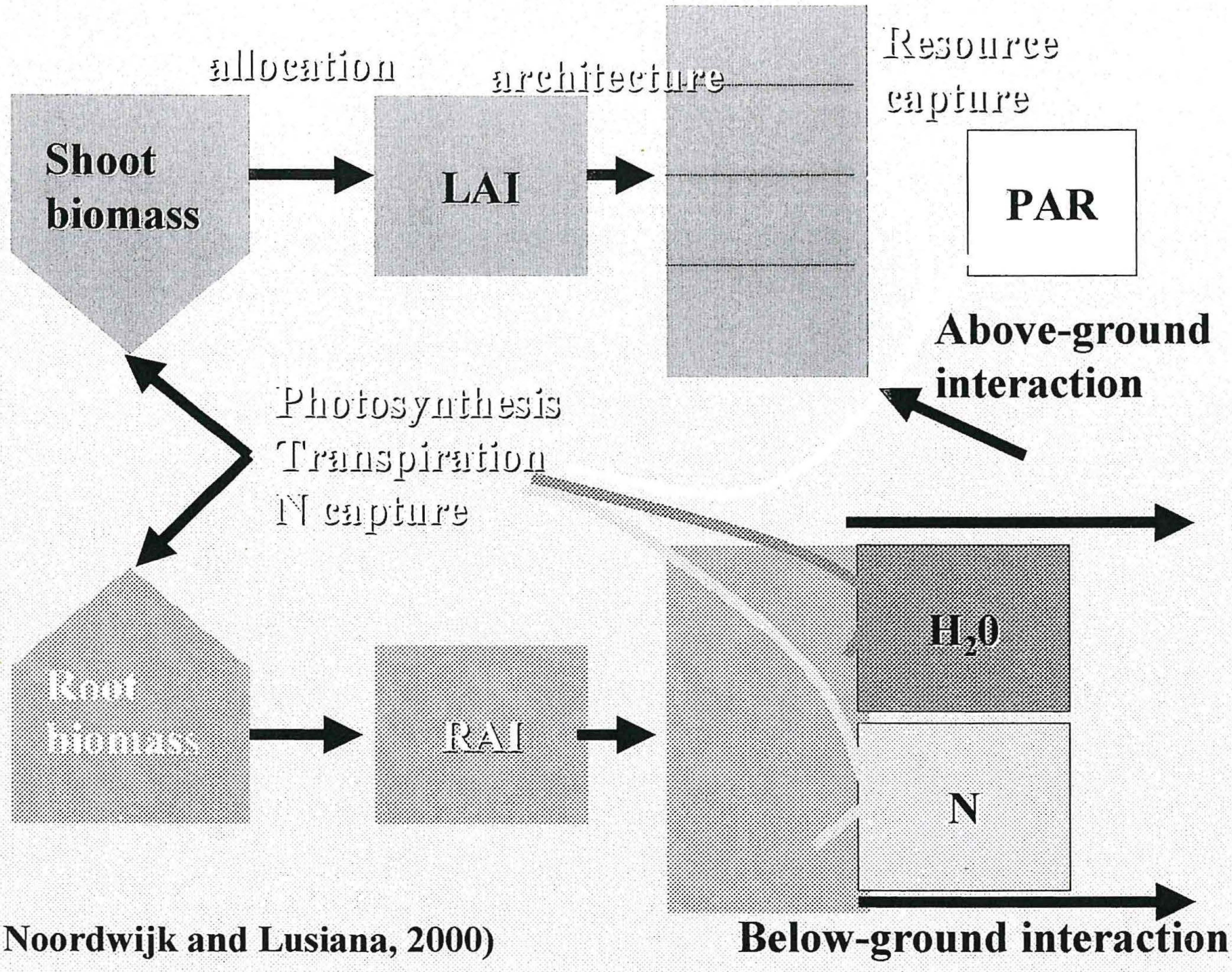
The main objectives of the WaNuLCAS model are: (quoted from WaNuLCAS 2.0 : Background on a model of Water, Nutrient and Light Capture in Agroforestry Systems, ICRAF, 2000)

1. integrate knowledge and hypotheses on below- and aboveground resource capture by trees and crops at patch scale as a basis for predicting complementary and competition

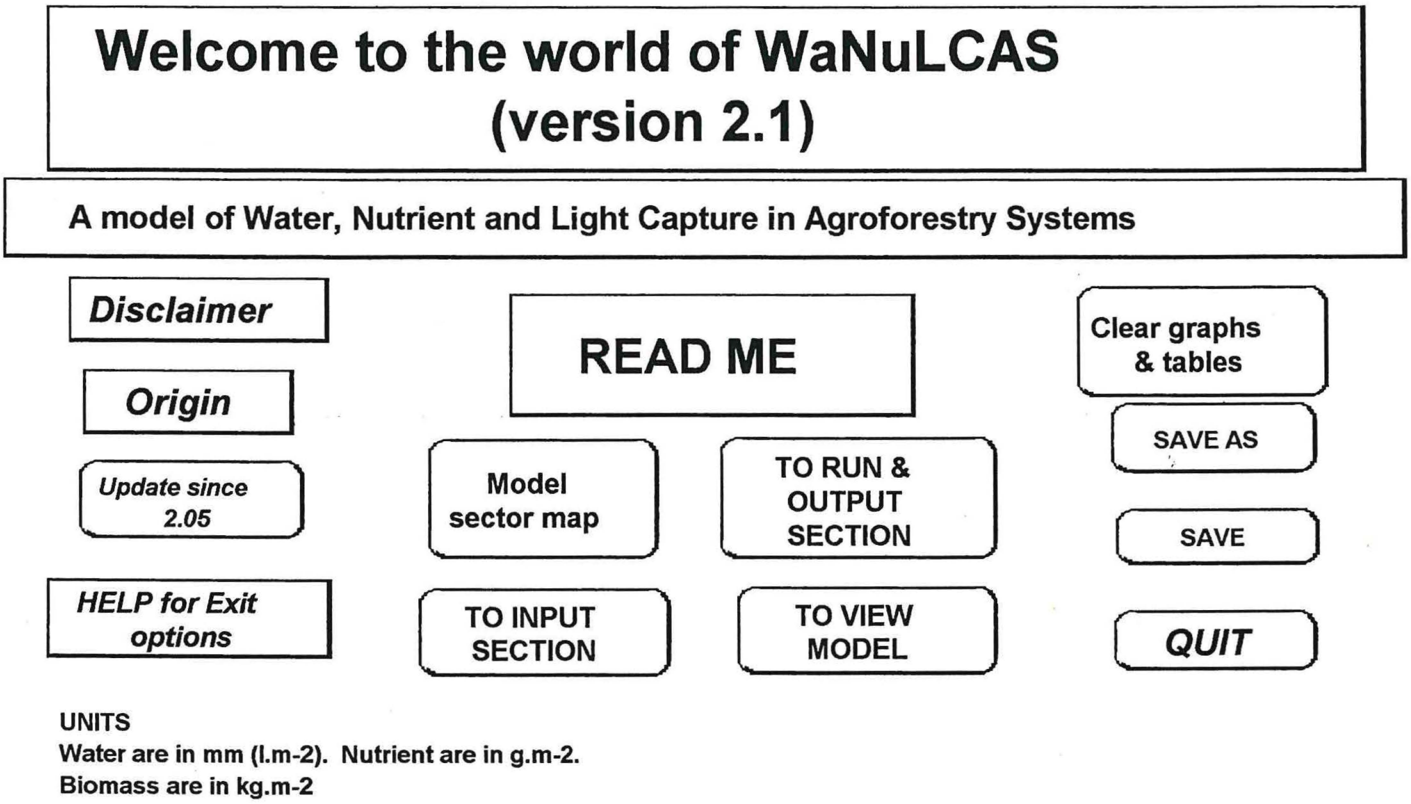
2. build on well-established modules (models) of a soil water, organic matter and nitrogen balance, and crop and a tree development to investigate interactions in resource capture

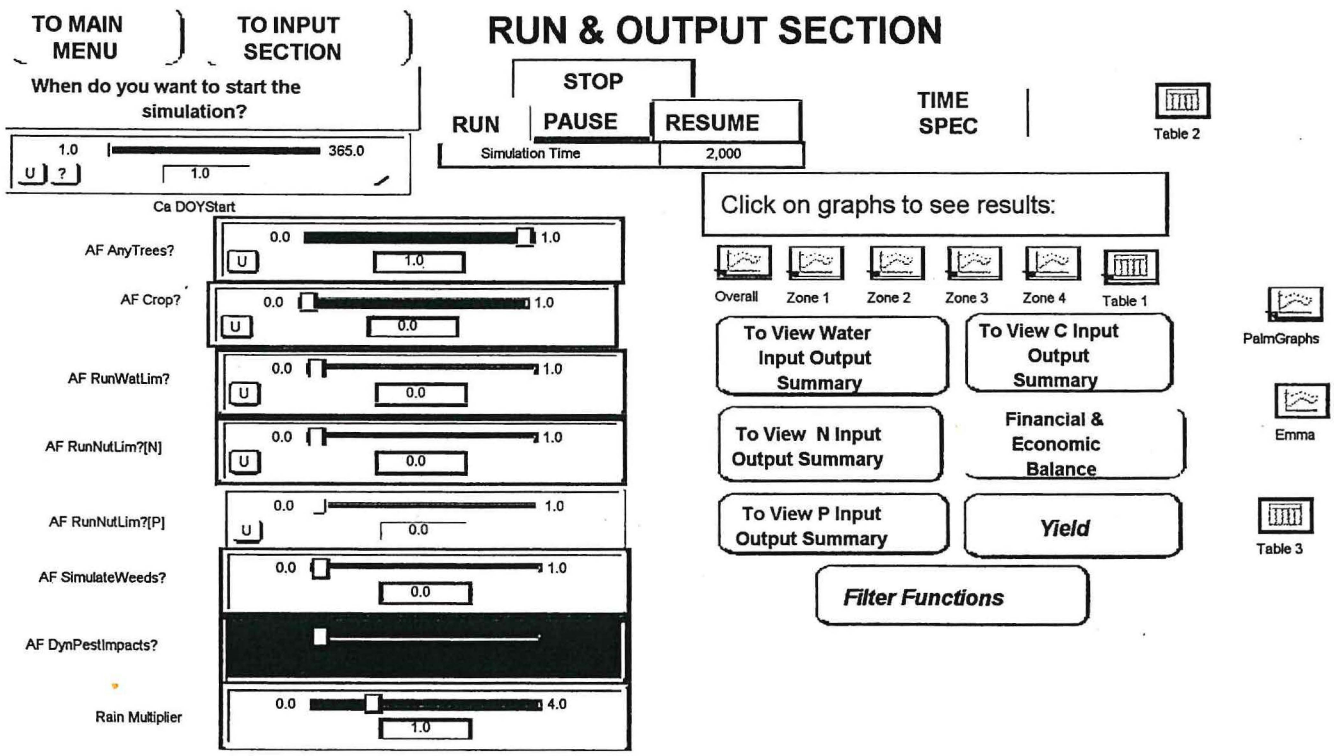
3. describe the plant-plant interaction term as the outcome of resource capture efforts by the components species, as determined by their above- and below ground architecture (spatial organisation) as well as physiology.

4. Be applicable to spatially zoned agroforestry system as well as rotational systems,



(van Noordwijk and Lusiana, 2000)


Welcome to the world of WaNuLCAS
(version 2.1)
A model of Water, Nutrient and Light Capture in Agroforestry Systems
Disclaimer
Origin
Update since 2.05
HELP for Exit options
READ ME
Model sector map
TO RUN & OUTPUT SECTION
TO INPUT SECTION
TO VIEW MODEL
Clear graphs & tables
SAVE AS
SAVE
QUIT
UNITS
Water are in mm (l.m-2). Nutrient are in g.m-2.
Biomass are in kg.m-2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TO INPUT SECTION
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AF AnyTrees?
AF Crop?
AF RunWatLim?
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AF RunNutLim?[P]
AF SimulateWeeds?
AF DynPestImpacts?
Rain Multiplier
0.0
4.0
RUN & OUTPUT SECTION
STOP
RUN
PAUSE
RESUME
Simulation Time
2,000
TIME SPEC
Table 2
Click on graphs to see results:
Overall
Zone 1
Zone 2
Zone 3
Zone 4
Table 1
PalmGraphs
To View Water Input Output Summary
To View C Input Output Summary
Emma
To View N Input Output Summary
Financial & Economic Balance
To View P Input Output Summary
Yield
Table 3
Filter Functions

TO MAIN MENU | TO INPUT LIST | HELP

Soil Temperature

Soil Temperature Type

1 — 3

? 1

Temp AType

Type 1 : constant temperature

15 — 40

U ? 24

Temp Cons

Type 2 : monthly average temperature

Temp MonthAvg

?

Type 3 : daily soil temperature

Soil Evaporation

Soil Evaporation Potential Type (0 = daily, 1 = consta

0.0 — 1.0

1.0

Temp PotEvapConst?

Constant temperature

Soil Evaporation

	Temp PotEvapConst	3

Daily soil evaporation potential

TO MAIN MENU | TO INPUT LIST

Initial Soil Water | Inaccessible Water | Soil Hydraulic Properties

Hydraulic Lift ?

W Hyd?

0.0 — 1.0

0.0

Initial Soil Nutrient | Initial Immobile Nutrient | Macropore bypass flow

Nitrate Fraction | Adsorption Constant for N | Adsorption Constant for P

Diffusivity Coeficient | Nutrient mobilization | Nutrient deposition

5. Avoid where possible the use of parameters which can only be derived by fitting the model to empirical data sets and maximise the use of parameters which can be independently measured

6. Be flexible in exploring management options within each type of agroforestry system

7. Be useful in estimating extrapolation domains for proven agroforestry techniques, as regards soil and climate properties, as well as tree and crop architecture

8. Be user-friendly and allow 'non-modellers' to explore a range of options, while remaining open to improvement without requiring a complete overhaul of the model

9. Generate output which can be used in existing spreadsheets and graphical software

10. Make use of readily available and tested modelling software.

2. Model features

A key feature of the model is the description of uptake of water and nutrients (N and P) on the basis of root length densities of the tree and the crop, plant demand factors and the effective supply by diffusion at a given soil water content.

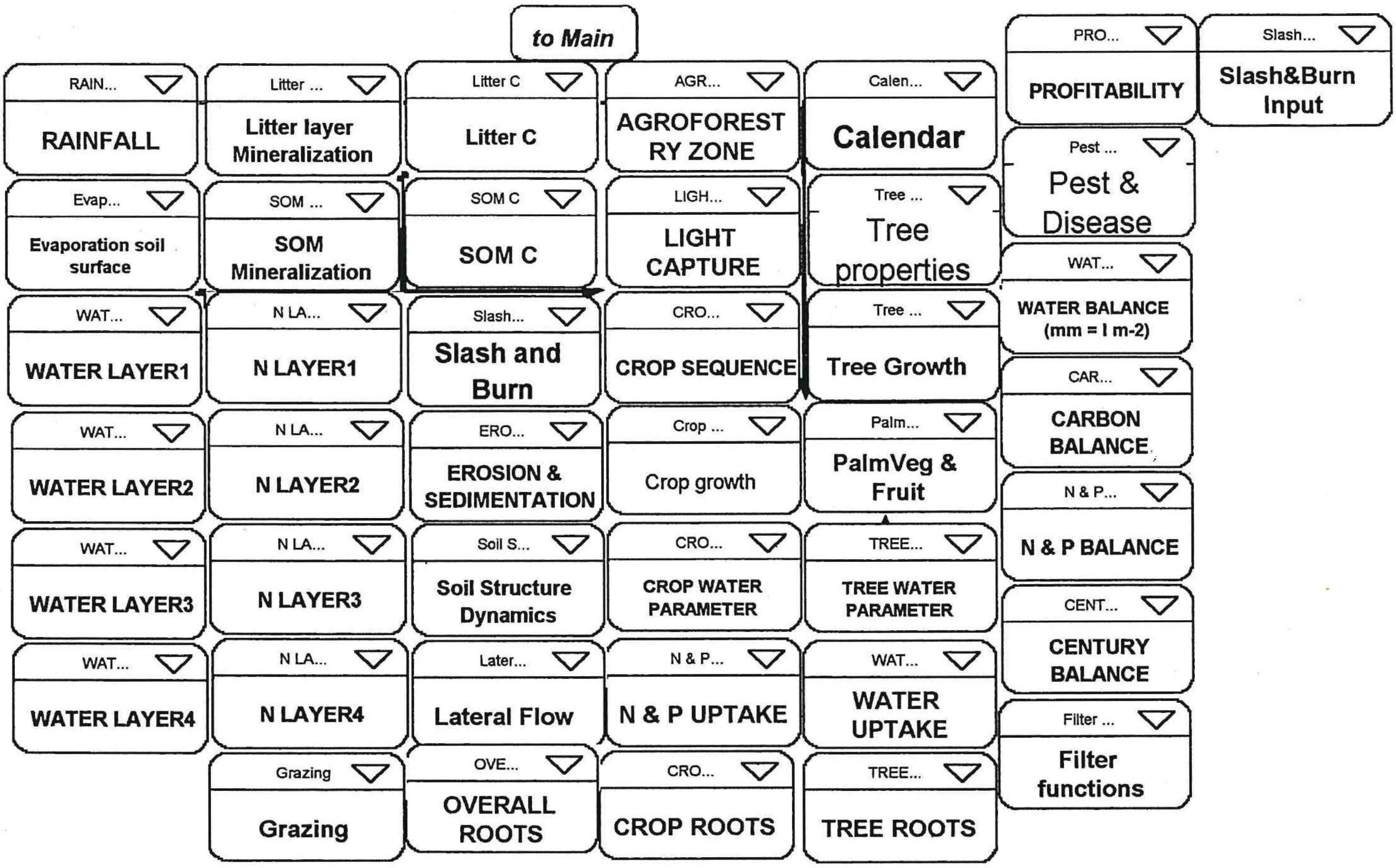
The model represent a four-layer soil profile, with four spatial zones, a water, nitrogen and phosphorus balance and uptake by a crop.

Climate effect is mainly included via daily rainfall data, which can be either read from a spreadsheet or generated on the basis of daily probability of rainfall.

Average temperature and radiation are reflected in the speed of phenological development.

Soil temperature is explicitly used as variable influencing decomposition and N and P mineralization.

Soil is represented in four layers, the depths of which can be chosen, with specified soil physical properties and initial water and nitrogen contents.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PROFITABILITY
Slash...
Slash&Burn Input
Evap...
Evaporation soil surface
SOM ...
SOM Mineralization
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SOM C
LIGH...
LIGHT CAPTURE
Tree ...
Tree properties
Pest ...
Pest & Disease
WAT...
WATER BALANCE (mm = l m-2)
WAT...
WATER LAYER1
N LA...
N LAYER1
Slash...
Slash and Burn
CRO...
CROP SEQUENCE
Tree ...
Tree Growth
CAR...
CARBON BALANCE
WAT...
WATER LAYER2
N LA...
N LAYER2
ERO...
EROSION & SEDIMENTATION
Crop ...
Crop growth
Palm...
PalmVeg & Fruit
N & P...
N & P BALANCE
WAT...
WATER LAYER3
N LA...
N LAYER3
Soil S...
Soil Structure Dynamics
CRO...
CROP WATER PARAMETER
TREE...
TREE WATER PARAMETER
CENT...
CENTURY BALANCE
WAT...
WATER LAYER4
N LA...
N LAYER4
Later...
Lateral Flow
N & P...
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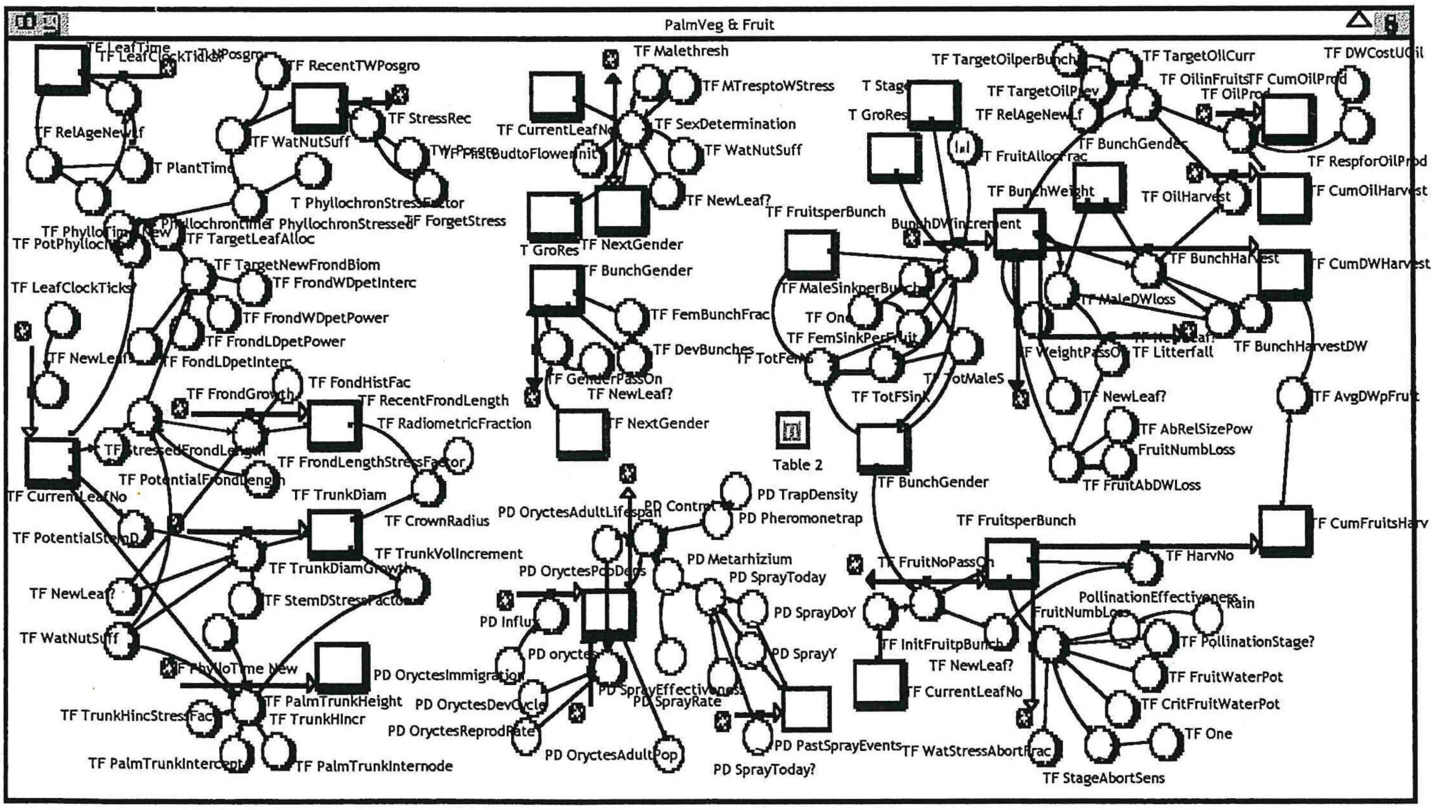
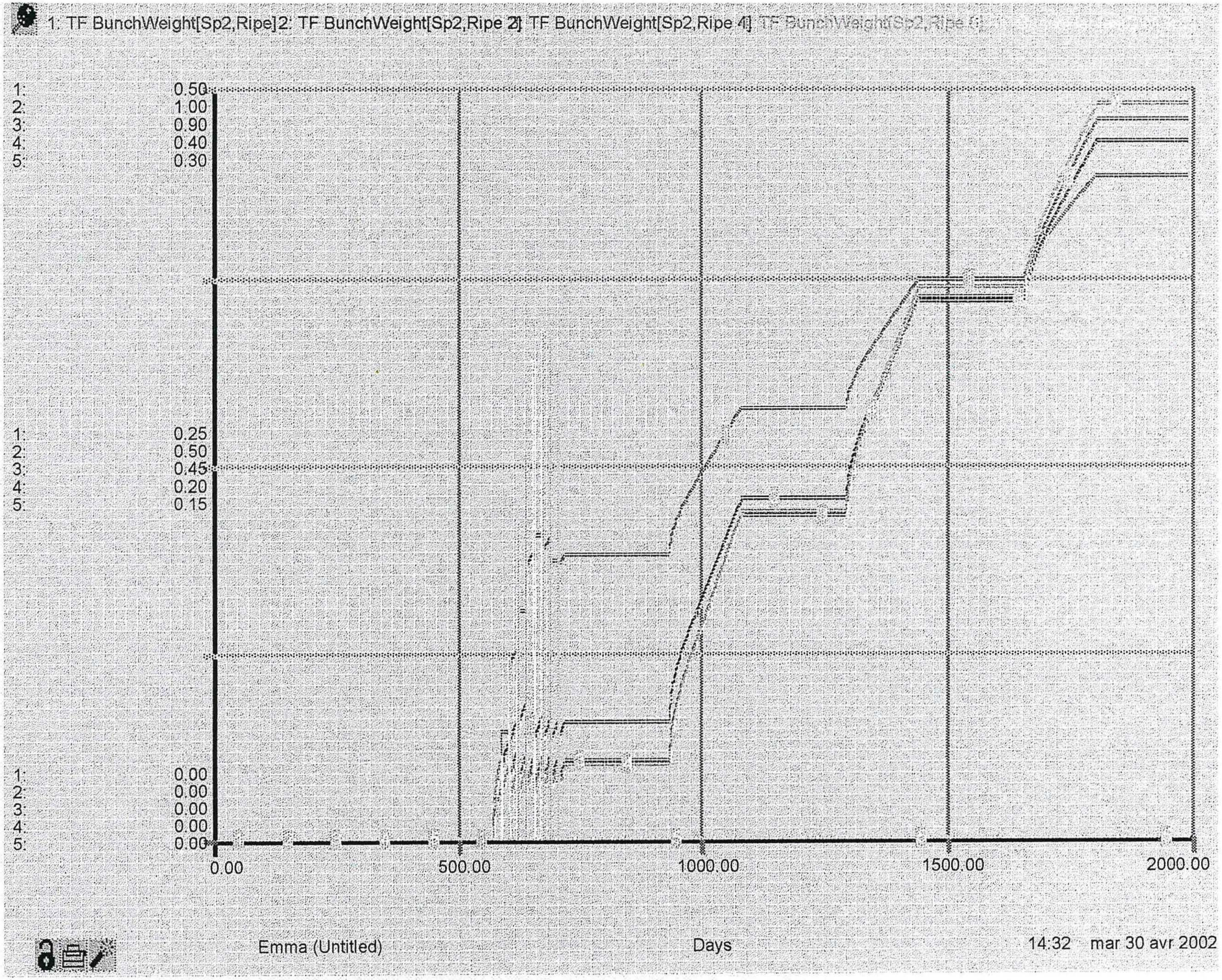
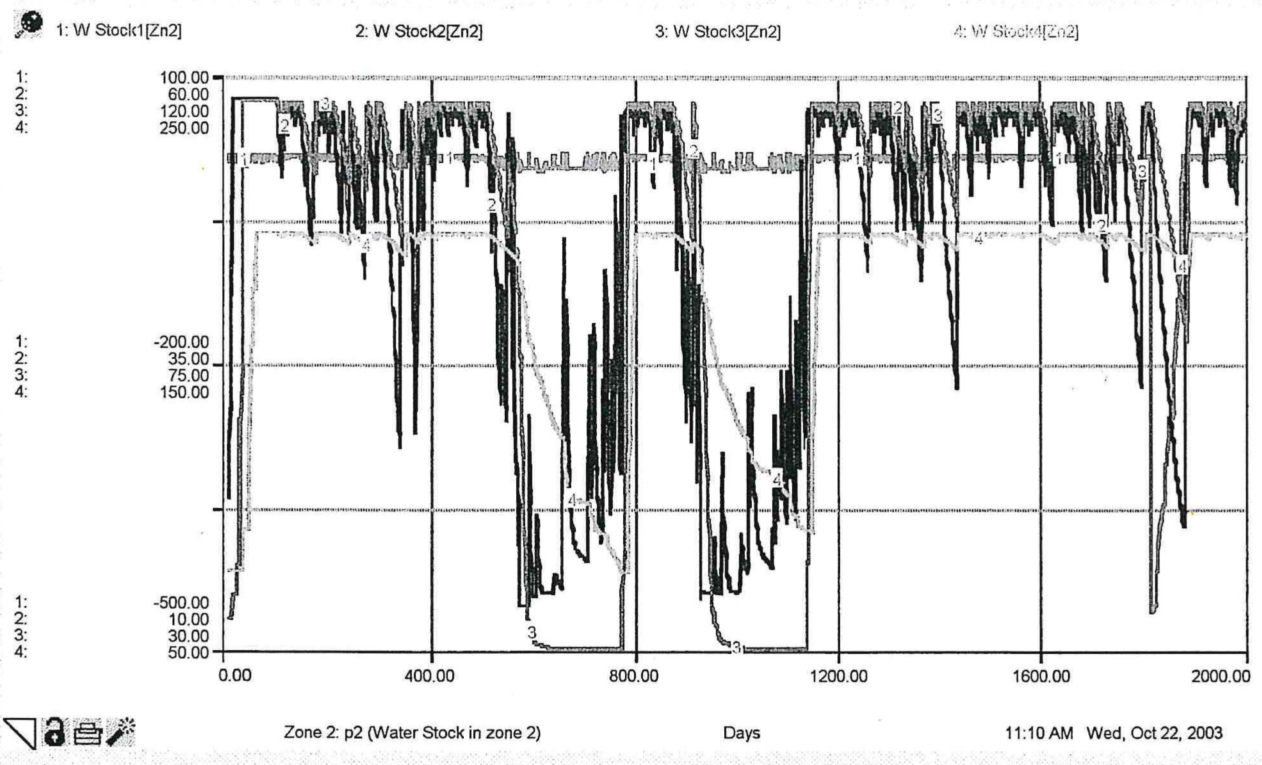
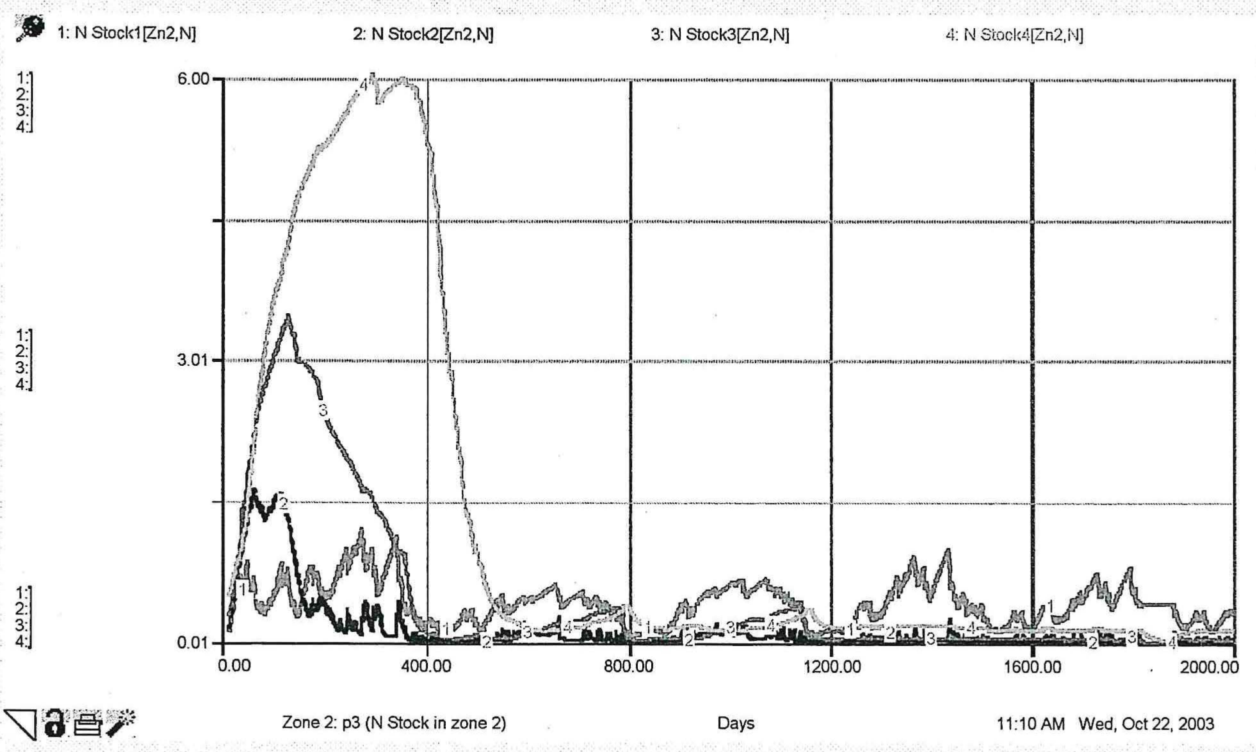
CROP ROOTS
TREE...
TREE ROOTS


PalmVeg & Fruit
TF LeafTime
TF LeafClockTicks
TF NPosgro
TF RecentTWPosgro
TF RelAgeNewLf
TF WatNutSuff
TF StressRec
T PlantTime
TF FirstBudtoFlowerInit
T PhyllochronStressFactor
TF PhylloTime
TF PotPhylloch
TF Phyllochrontime
TF PhyllochronStresses
TF ForgetStress
TF TargetLeafAlloc
TF TargetNewFrondBiom
TF FrondWDpetInterc
TF LeafClockTicks
TF FrondWDpetPower
TF FrondLDpetPower
TF NewLeaf?
TF FondLDpetInterc
TF FondHistFac
TF FrondGrowth
TF RecentFrondLength
TF RadiometricFraction
TF StressedFrondLength
TF FrondLengthStressFactor
TF PotentialFrondLength
TF CurrentLeafNo
TF TrunkDiam
TF CrownRadius
TF PotentialStemD
TF TrunkVolIncrement
TF TrunkDiamGrowth
TF NewLeaf?
TF StemDStressFactor
TF WatNutSuff
TF PhylloTime New
TF PalmTrunkHeight
TF TrunkHincStressFac
TF TrunkHIncr
TF PalmTrunkIntercept
TF PalmTrunkInternode
TF Malethresh
TF MTresptoWStress
TF CurrentLeafNo
TF SexDetermination
TF WatNutSuff
TF NewLeaf?
T GroRes
TF NextGender
TF BunchGender
TF FemBunchFrac
TF DevBunches
TF GenderPassOn
TF NewLeaf?
TF NextGender
Table 2
PD TrapDensity
PD OryctesAdultLifespan
PD Control
PD Pheromonetrap
PD OryctesPopDens
PD Metarhizium
PD SprayToday
PD SprayDoY
PD Influx
PD oryctes
PD SprayY
PD OryctesImmigration
PD SprayEffectiveness
PD SprayRate
PD OryctesDevCycle
PD OryctesReprodRate
PD OryctesAdultPop
PD PastSprayEvents
PD SprayToday?
TF TargetOilperBunch
TF TargetOilCurr
TF DWCostUOil
T Stage
TF TargetOilPrev
TF OilinFruits
TF CumOilProd
T GroRes
TF RelAgeNewLf
TF OilProd
TF FruitAllocFrac
TF BunchGender
TF RespforOilProd
TF BunchWeight
TF OilHarvest
TF CumOilHarvest
TF FruitsperBunch
BunchDWincrement
TF BunchHarvest
TF CumDWHarvest
TF MaleSinkperBunch
TF MaleDWloss
TF One
TF FemSinkPerFruit
TF TotFem
TF WeightPassOn
TF Litterfall
TF BunchHarvestDW
TF TotMaleS
TF TotFSink
TF NewLeaf?
TF AvgDWpFruit
TF AbRelSizePow
TF FruitNumbLoss
TF FruitAbDWLoss
TF BunchGender
TF FruitsperBunch
TF CumFruitsHarv
TF FruitNoPassOn
TF HarvNo
PollinationEffectiveness
Rain
FruitNumbLoss
TF InitFruitpBunch
TF PollinationStage?
TF NewLeaf?
TF FruitWaterPot
TF CurrentLeafNo
TF CritFruitWaterPot
TF One
TF WatStressAbortFrac
TF StageAbortSens



Ouput from WaNuLCAS for oil palm


1: W Stock1[Zn2]
2: W Stock2[Zn2]
3: W Stock3[Zn2]
4: W Stock4[Zn2]
1: 100.00
2: 60.00
3: 120.00
4: 250.00
1: -200.00
2: 35.00
3: 75.00
4: 150.00
1: -500.00
2: 10.00
3: 30.00
4: 50.00
0.00
400.00
800.00
1200.00
1600.00
2000.00
Zone 2: p2 (Water Stock in zone 2)
Days
11:10 AM Wed, Oct 22, 2003


1: N Stock1[Zn2,N]
2: N Stock2[Zn2,N]
3: N Stock3[Zn2,N]
4: N Stock4[Zn2,N]
6.00
3.01
0.01
0.00
400.00
800.00
1200.00
1600.00
2000.00
Zone 2: p3 (N Stock in zone 2)
Days
11:10 AM Wed, Oct 22, 2003

The water balance of the system includes rainfall and canopy interception, with the option of exchange between the four zones by run-on and run-off as well as subsurface lateral flows, surface evaporation, uptake by the crop and tree and leaching.

Growth of both plants (crop and tree) is calculated on a daily basis by multiplying potential growth with the minimum of three stress factors.

3. Adaptation of WaNulCAS for alternative oil palm production systems

The Malaysian Palm Oil Board with ICRAF has started a research programm on the adaptation of the WaNuLCAS platform for alternative oil palm production systems.

They presented this manuscript :

"Adapting a generic tree-soil-crop interaction model based on light, water and nutrient capture for the evaluation of current and alternative oil palm production systems"

by Meine van Noordwijk, Mohammed Haniff Harun and Chan Kook Weng, PIPOC 20-22 August 2001.

Abstract :

The paper describe a new module representing the physiology and phenology of palm fruit development for the generic tree-soil-crop interaction model WaNuLCAS. The combined model can be used for evaluating scenarios of land use conversion from forest or degraded lands into oil palm in combination with other crops or trees. Key to the sucess of such model is the way influences of past stress episodes on the development of fruit bunches is described.

Hypothesis (model elements) for oil palm sexualisation in this model are : (quoted from the manuscript)

- phyllochron time keeping : the time interval between the emergence of two leaves or phyllochron, determines the basic unit of time for floral and fruit development as well. For current oil palm germplasm a phyllochron is about 14 days.
- Sex determination of flowers is related to the internal condition of the palm, based on its internal growth reserves, as well as in response to current water stress; as in the model the switch between male and female bunches is set at a single day, we do it on the basis of a moving average of the past water stress levels; because of the link with internal growth reserves the module can account for the tendency to male flowers caused by tapping for palm wine, as is common practice in W. Africa.
- Simultaneous development and resource competition between the various male and female flowers and bunches present at any point in time, with an age- dependent relative sink strength,
- Abortion of individual fruits in a bunch, in response to water stress
- Harvest of one (potential) bunch at the end of each phyllochron unit

Short conclusion

Nowadays in Asia, oil palm is major contributor on both **aforestation** and **reforestation** : it is of prime importance to study the effect of this tree crop on environment (sustainability criteria) as well as the effect of environment on its development (functional ecology). It is the reason why based of previous citation, three different levels of research investigations will be undertaken.

The first one deal with the **ecological impact** of oil palm production in Sumatra. This can include surveys about the decrease of biodiversity, the overuse of natural ressource as water and nutrients, aforestation, and the environmental pollution of palm oil factories with as a main target to propose guidelines throught "impact indicators" lists.

The second one will deal the analyse of **the effect of environment** including global change on oil palm growth and development.

The third aspect may concerned the **ecological contribution** of oil palm to decrease the green house gas in the atmosphere (carbon sequestration) and the soil erosion.

The development of new investigations tools as **natural isotopes**, "biological tracer" in the plant, for a better understanding of its internal functionning is one example. Some possible applications could be in the identification of new well adapted progenies to marginal zones.

At least , some simulations of cropping system based on oil palm with **multi-agent system platform** (WaNuLCAS) and the elaboration of scenarii could improve the agronomic and economic managment of this crop.

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