A tree under stress
Ecophysiology of the rubber tree
Hevea brasiliensis

Philippe Thaler & Régis Lacote

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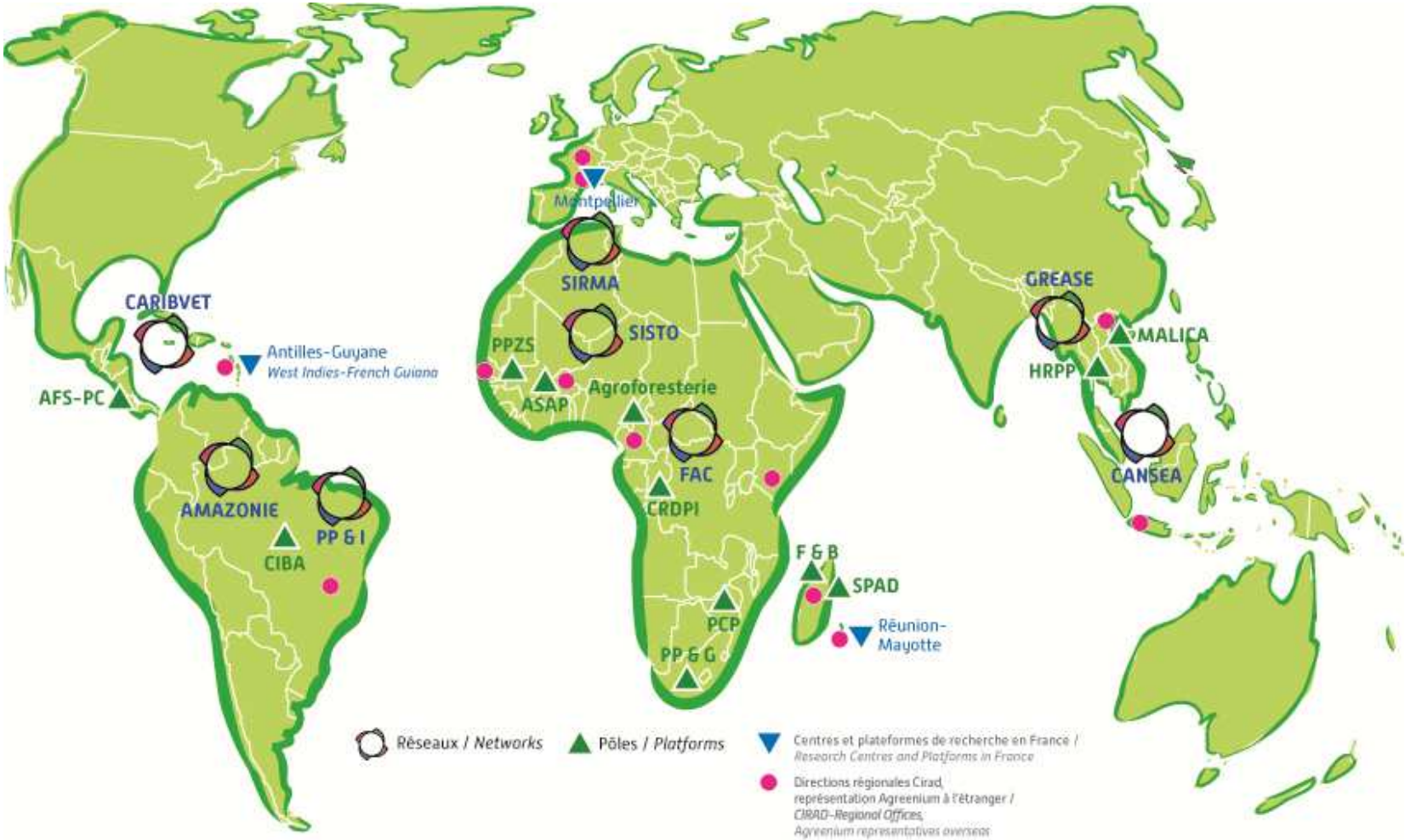
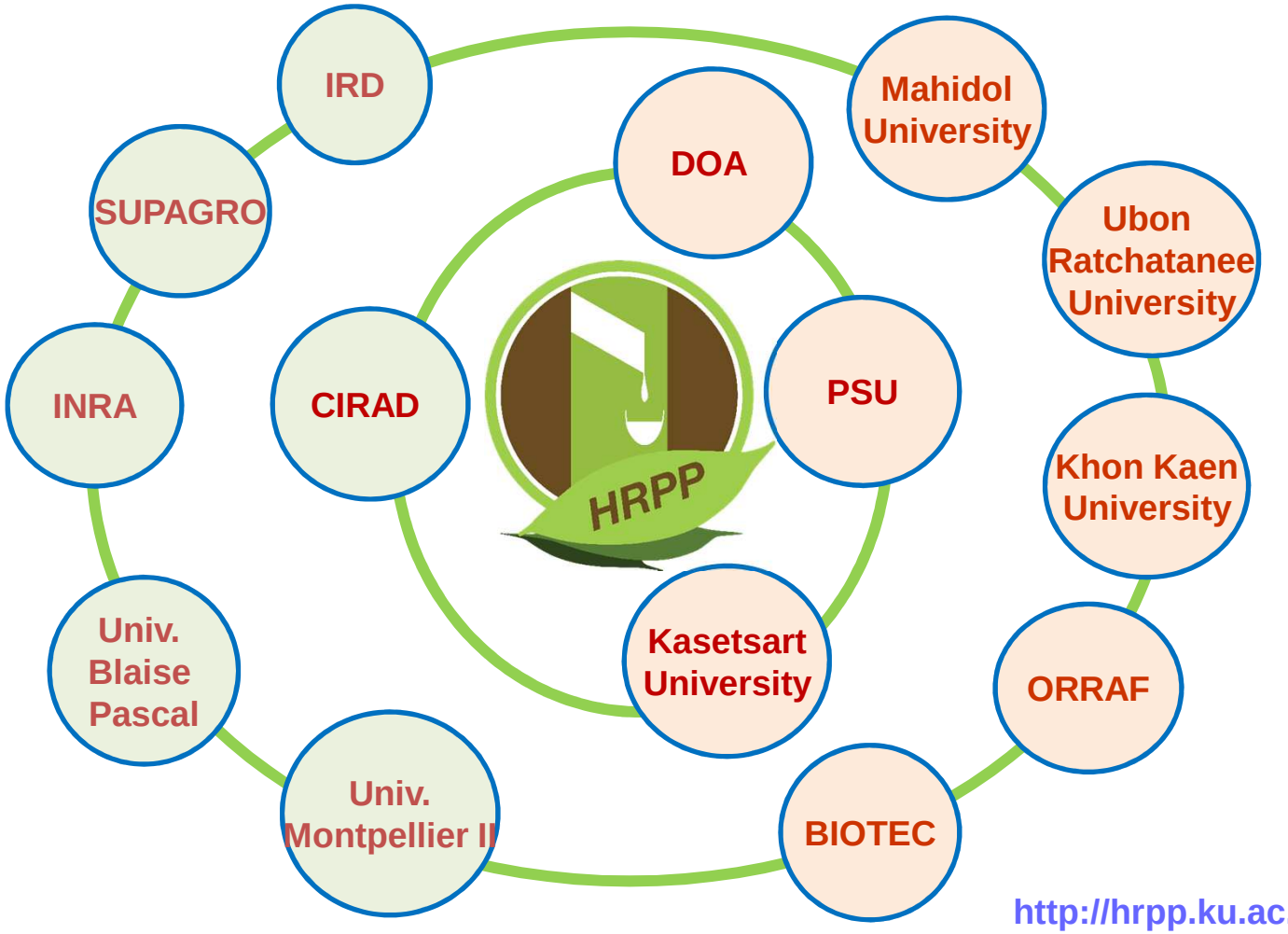
Working together for tomorrow's agriculture

Priority research initiatives in partnership
Research platforms



Hevea Research Platform in Partnership: a network of *14* research and higher education *institutions* from *Thailand* and *France*



http://hrpp.ku.ac.th

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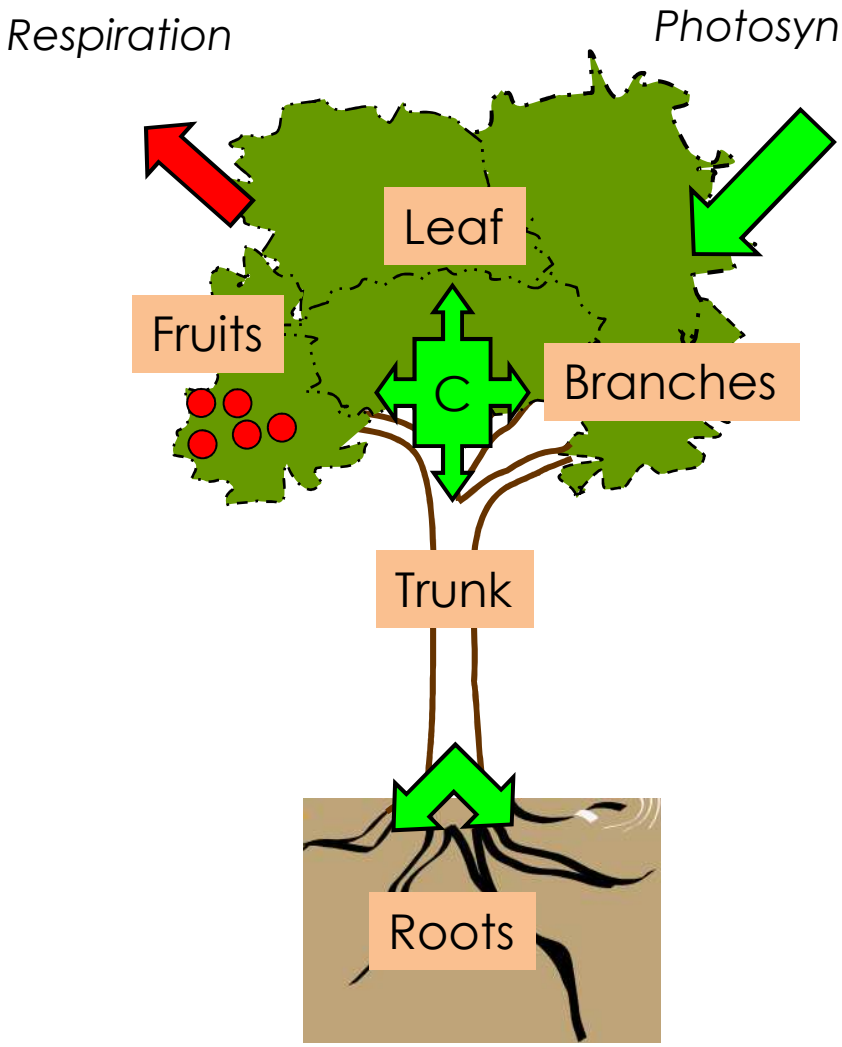
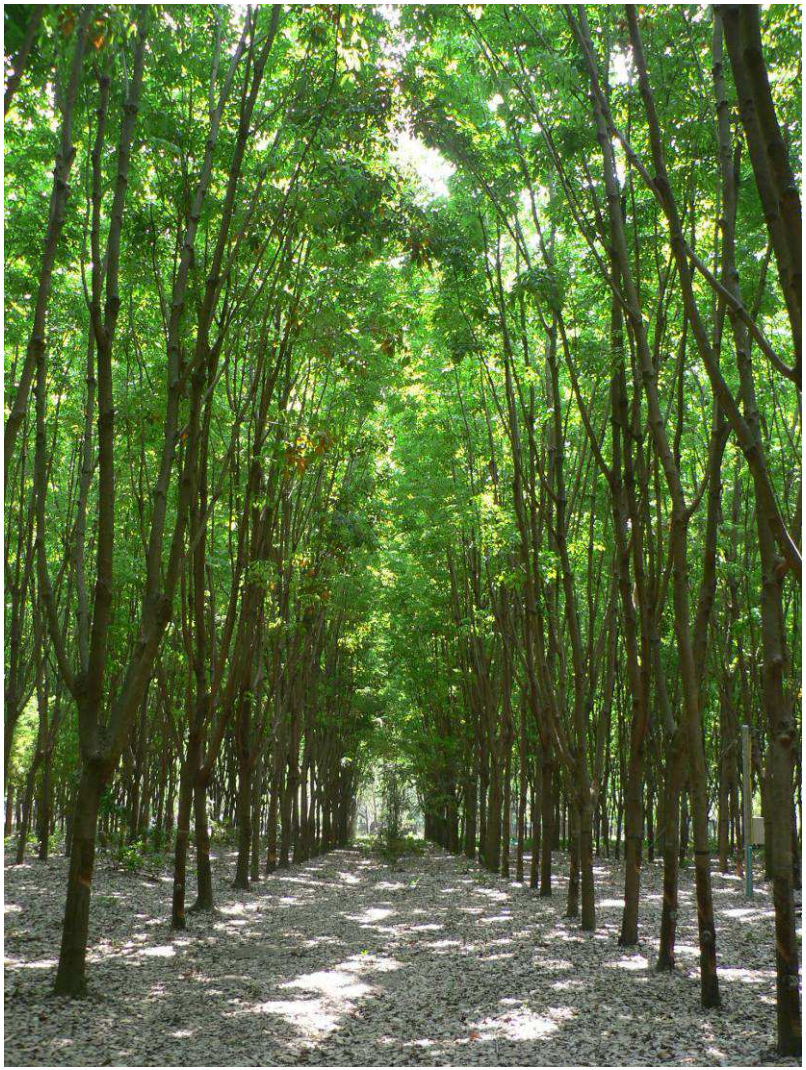
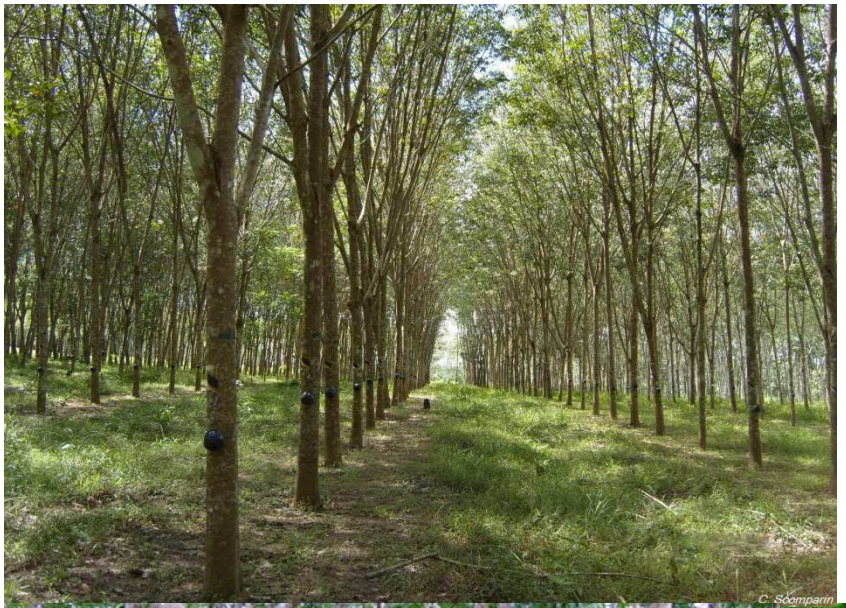
Dynamics of carbon resources in trees



- What are the drivers of the allocation of carbohydrate resources in trees?
- How to model processes of allocation, growth and development as a function of climate?
- How to link tree structure to its functions (acquisition and allocation of resources)?

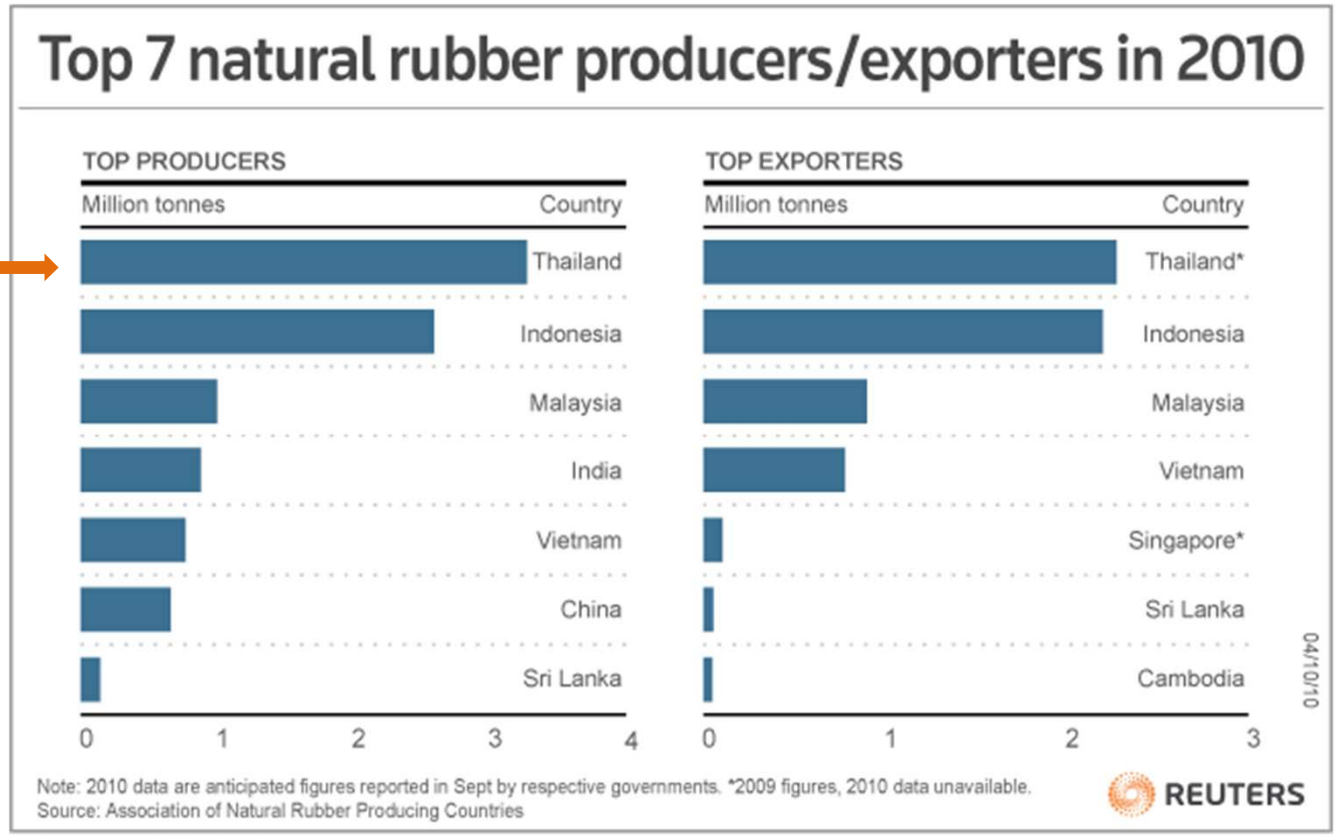


Why is rubber tree so interesting?



The typical landscape in southern Thailand

A major economic crop, worldwide and particularly in Thailand



Reuters graphic/Christine Chan

Thailand: N°2 in planted area, but **N°1** in production (3.35 Mt in 2011) and exports.
About **6 millions** of Thai people make a living from NR production or NR industry!

A very specific product and way of exploitation

Exploitation by tapping

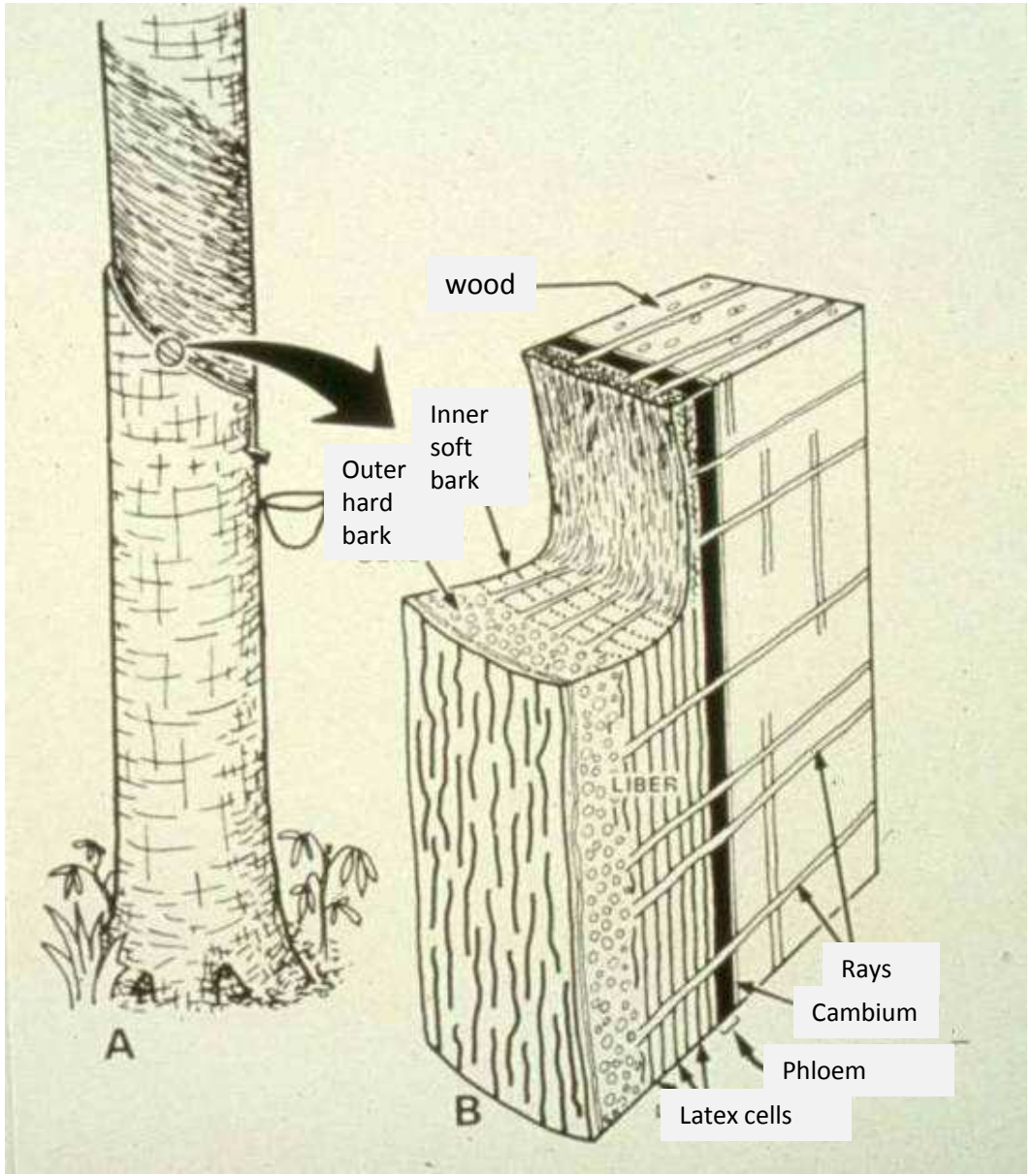
Shaving of the bark of the tree with the use of a tapping knife induce latex flow.
Extraction of the **latex** cell cytoplasm, which is not the sap of the tree.

- The most important practice determining production is then harvest itself (tapping).
- Untapped trees do not regenerate latex!
- Natural rubber (poly-isoprene) accounts for about 90 % of latex d.m.
- Induction of an **artificial C sink**, competing with other functions, as unttaped trees do not export latex

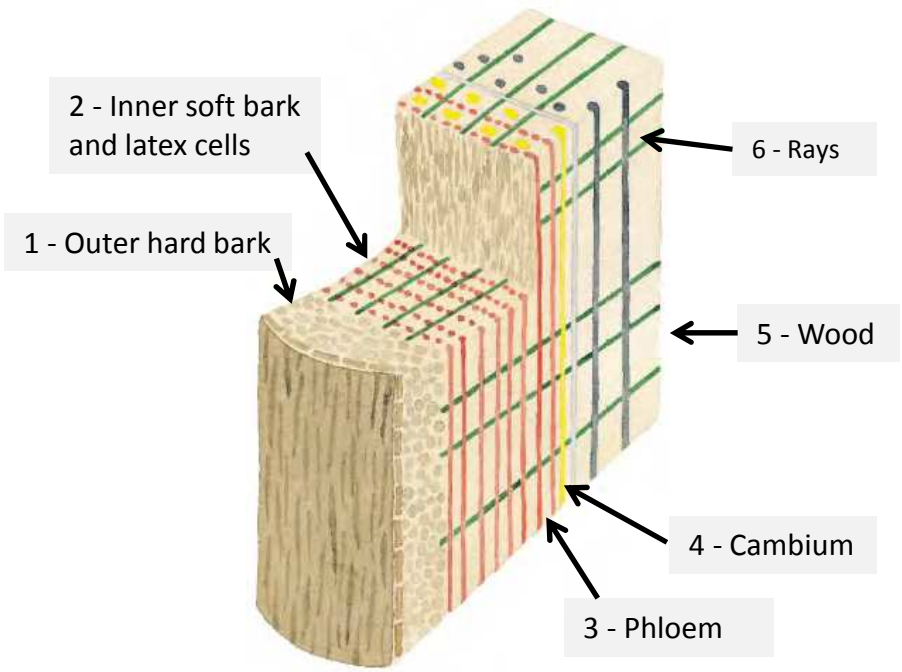
Particularities of the rubber yield

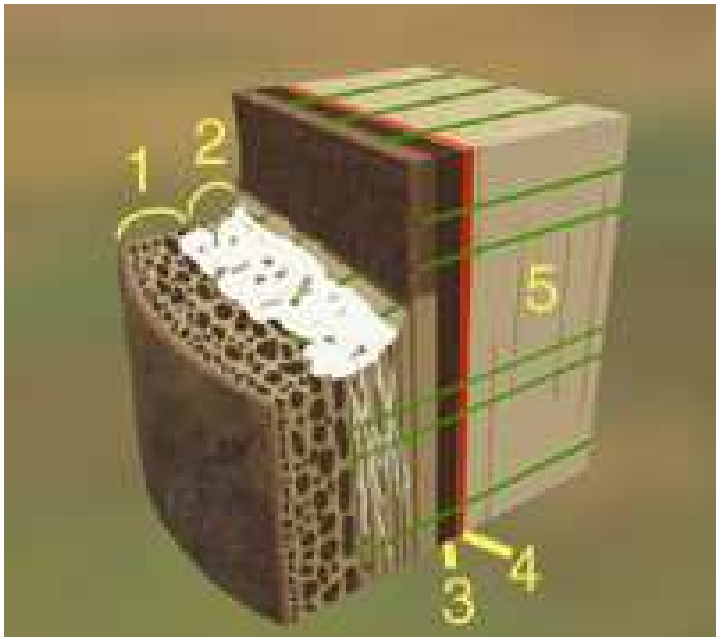
Bark anatomy

The laticifers



Particularities of the rubber yield

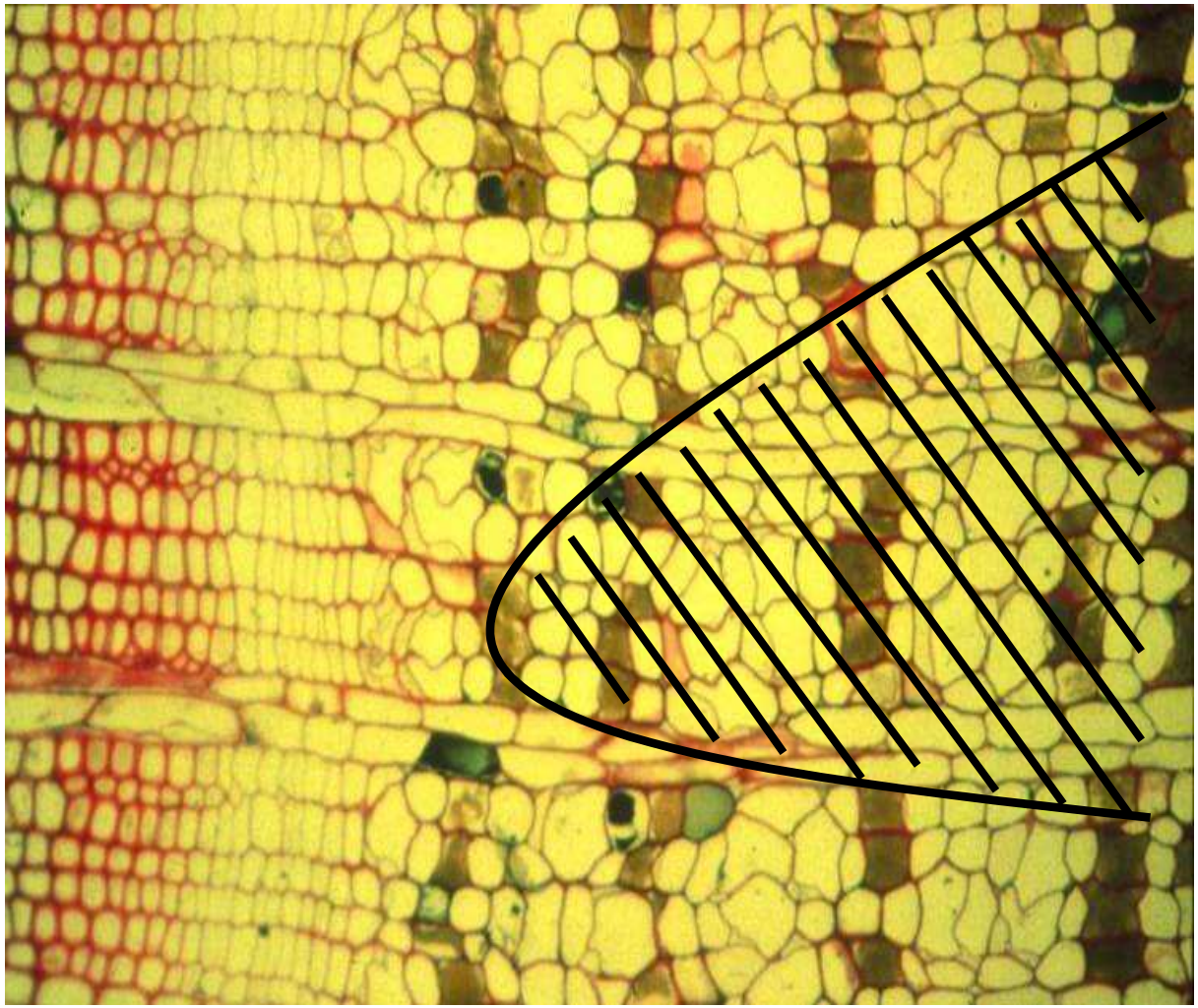




Bark anatomy: the laticifers

Hebant et de Fay 1981
De Fay, Sanier et Hébant 1988

Particularities of the rubber yield



Particularities of the rubber yield

- Other cultures
  - **Organs vegetative/reproduction**
  - **re-produced de novo**
  - **apical meristems**
  - **naturally**
  - **sink for assimilates**
    - **Natural and large**

- Latex - NR
  - **Cytoplasm, specialized cells**
  - **regenerated in situ**
  - **cambium**
  - **artificially**
  - **sink for assimilates**
    - **Artificial and small**

Particularities of the rubber yield

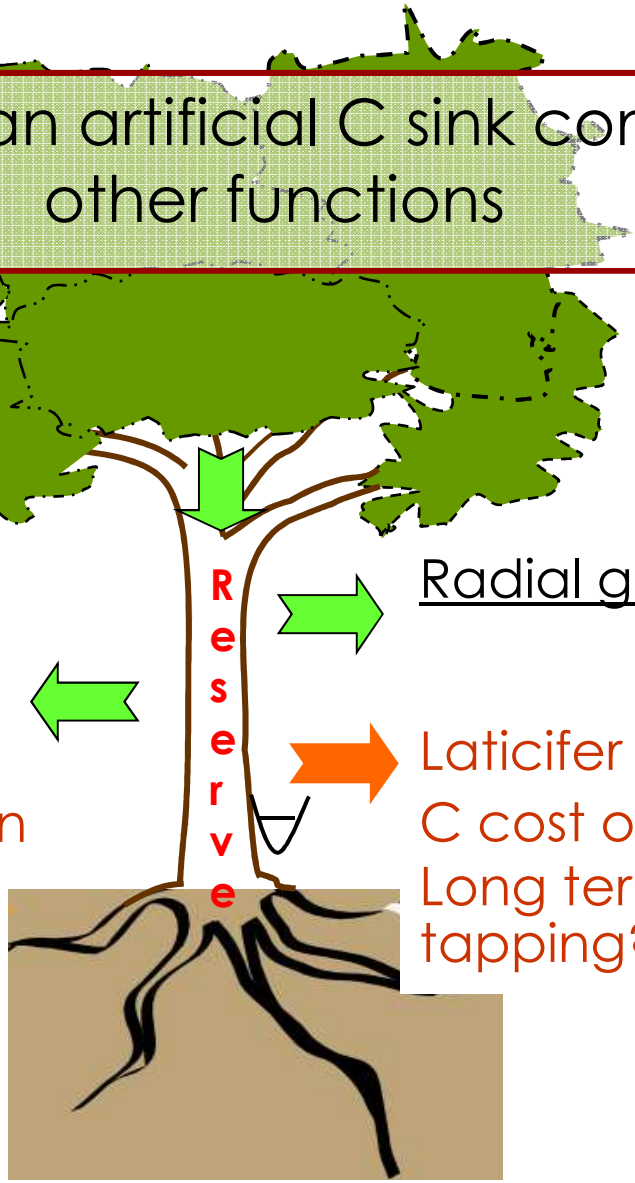
Tapping

A technical latex harvesting methods requiring training and skills: this is the human labor who makes the performance and guarantees with the quality of its work, the sustainability of plantations

Tapping school in Indonesia

Integrate the latex function into the whole tree functionning

Tapping creates an artificial C sink competing with other functions



Respiratory cost?

- Maintenance
- Growth
- Tapping/regeneration

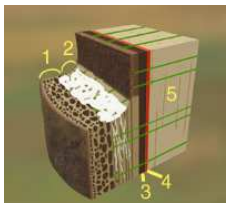
Radial growth

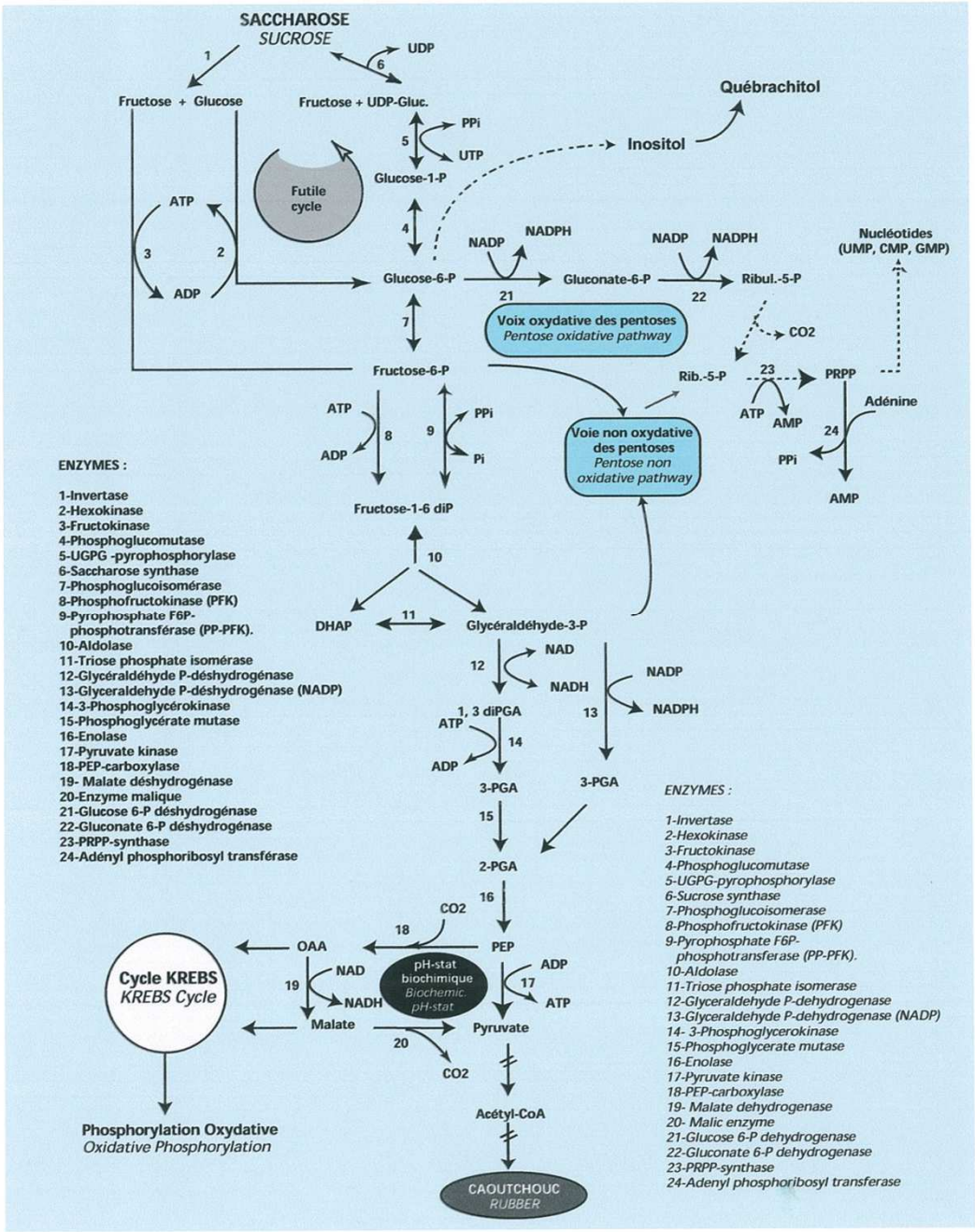
Laticifer system

C cost of tapping?

Long term Influence of tapping?

Metabolism of the latex cell: glycolysis and related reactions in latex cell

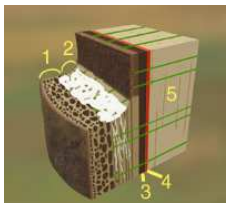


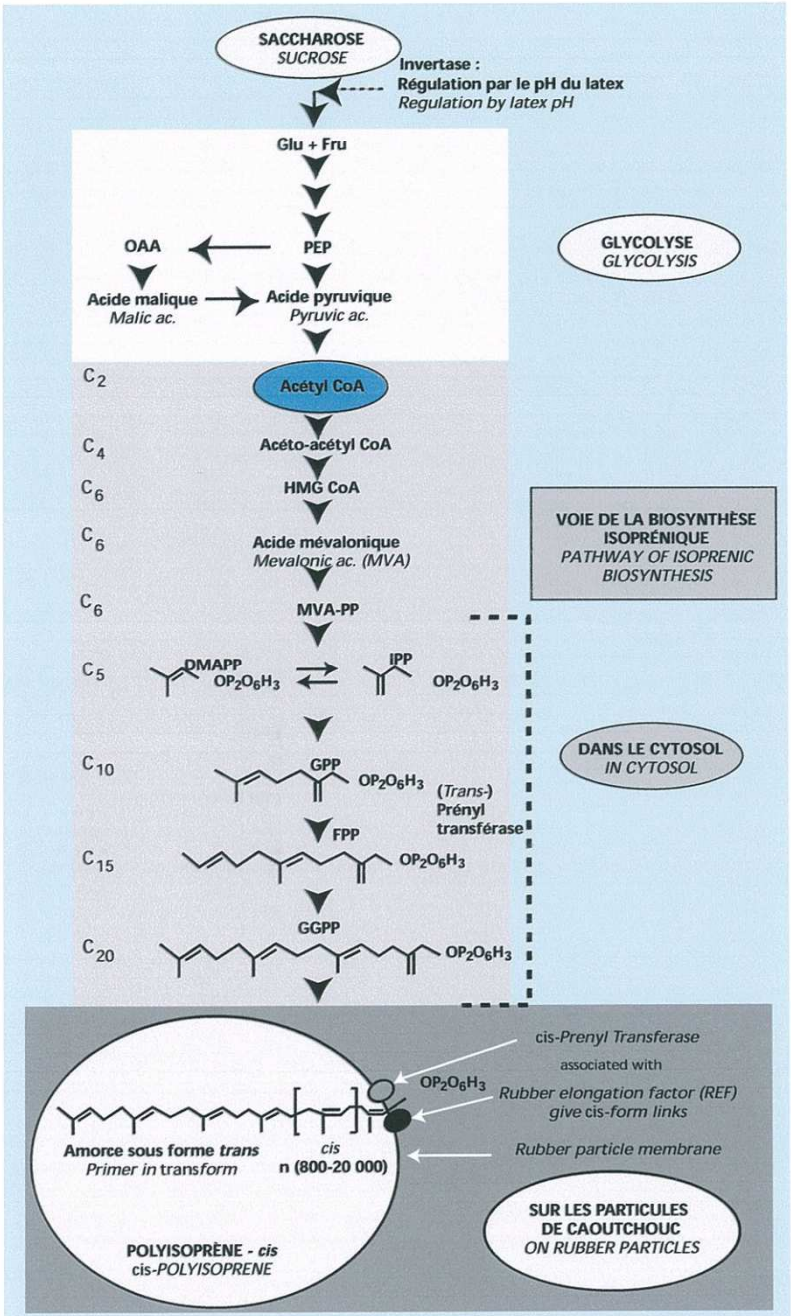


Jacob J.L, Prévôt J.C., Lacote R., Gohet E., Clément A., Gallois R.1, Joet T.1, Pujade-Renaud V.2, d'Auzac J. 1988

Particularities of the rubber yield

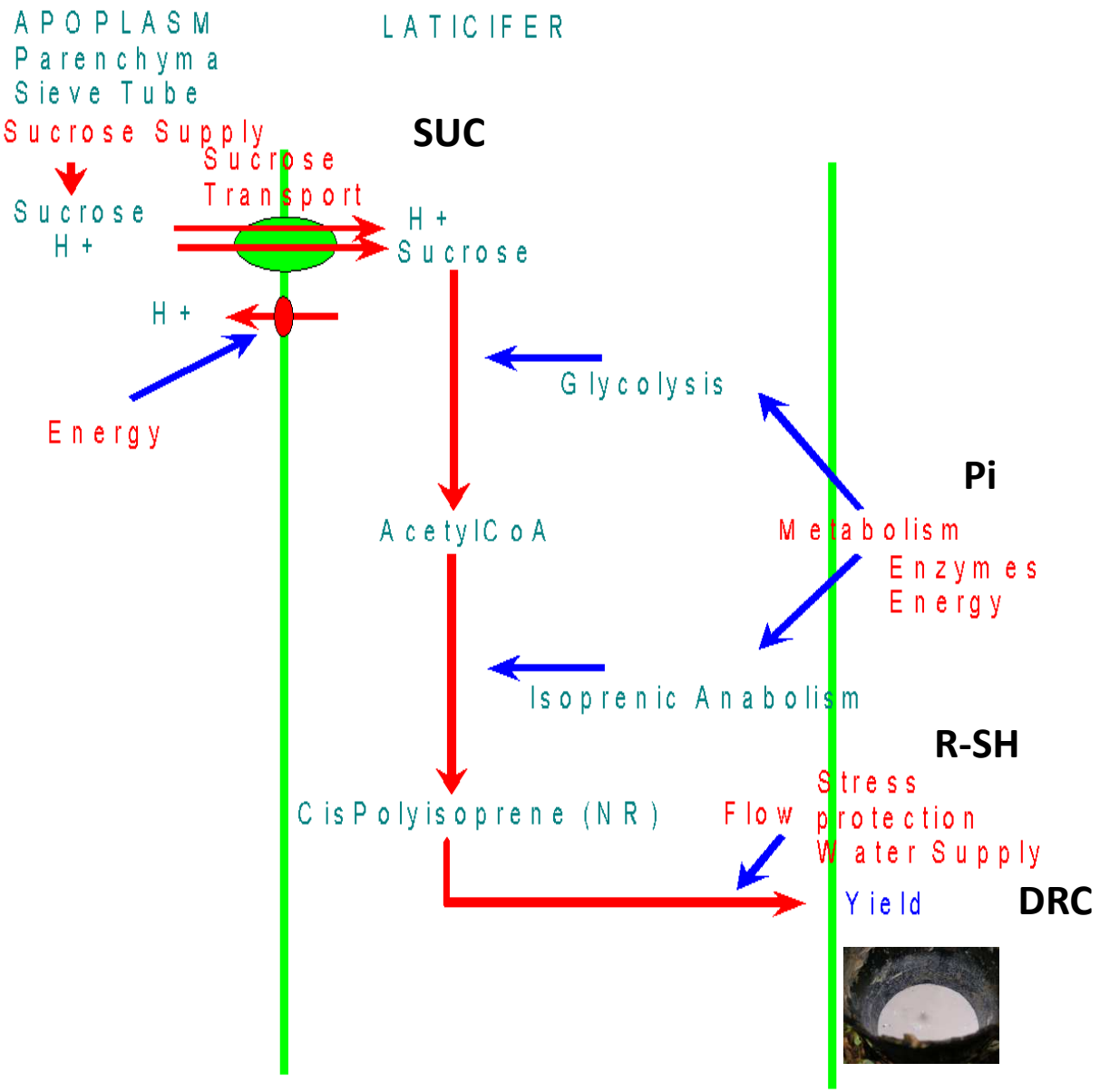
Metabolism of the latex cell: from sucrose to rubber





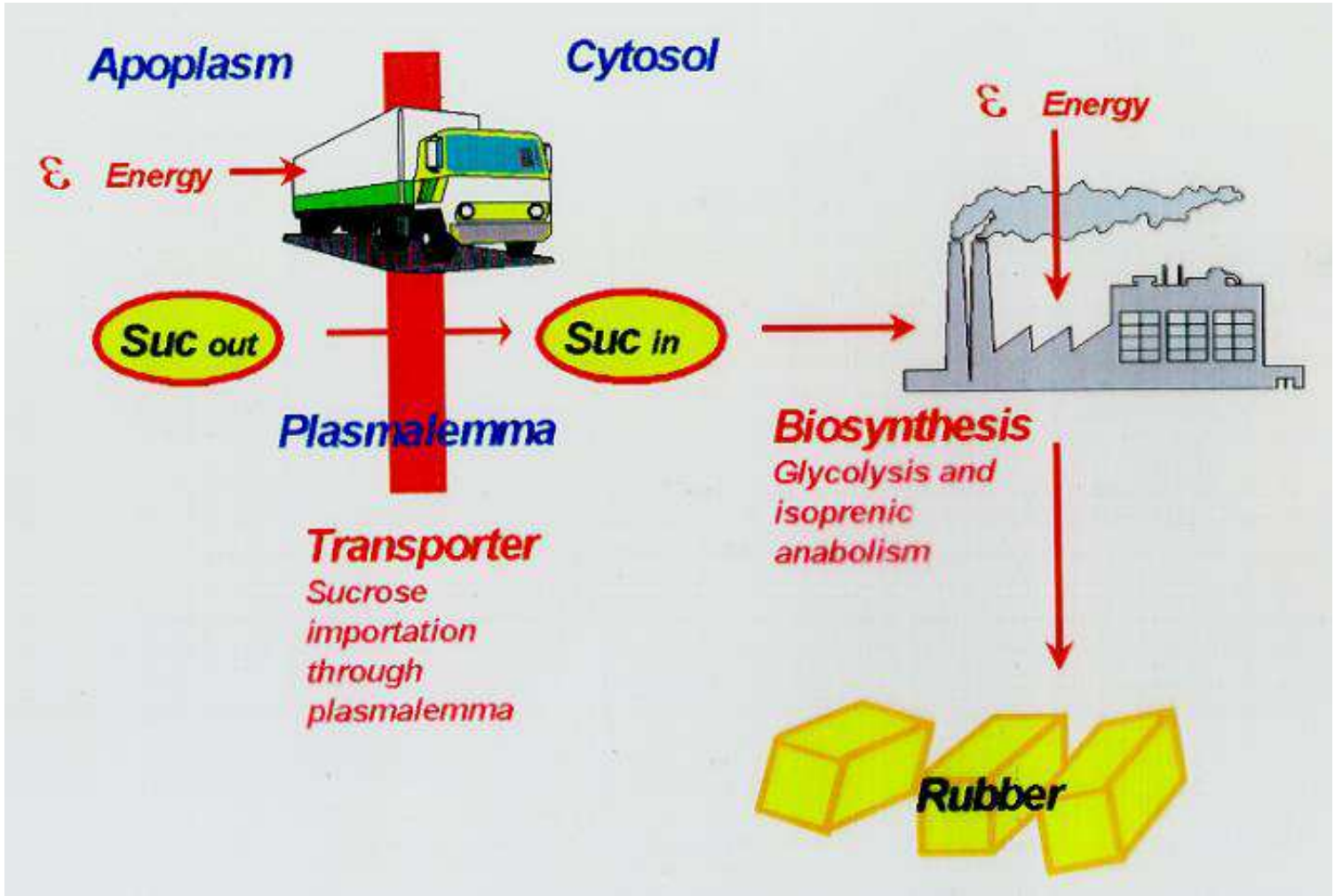
Jacob J.L, Prévôt J.C., Lacote R., Gohet E., Clément A., Gallois R.1, Joet T.1, Pujade-Renaud V.2, d'Auzac J. 1988

Latex cell metabolism



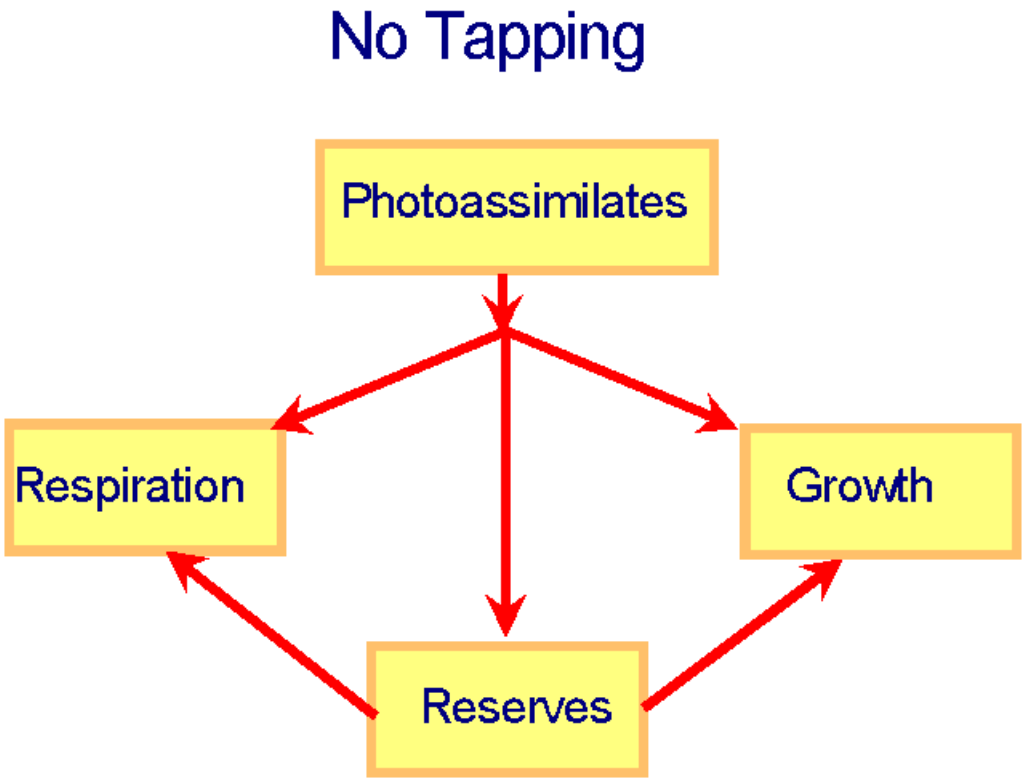
Latex cell metabolism

Carbon, sucrose, energy, a green rubber factory



Tapping and latex harvesting

- Tapping the rubber tree modifies the natural metabolism of the tree



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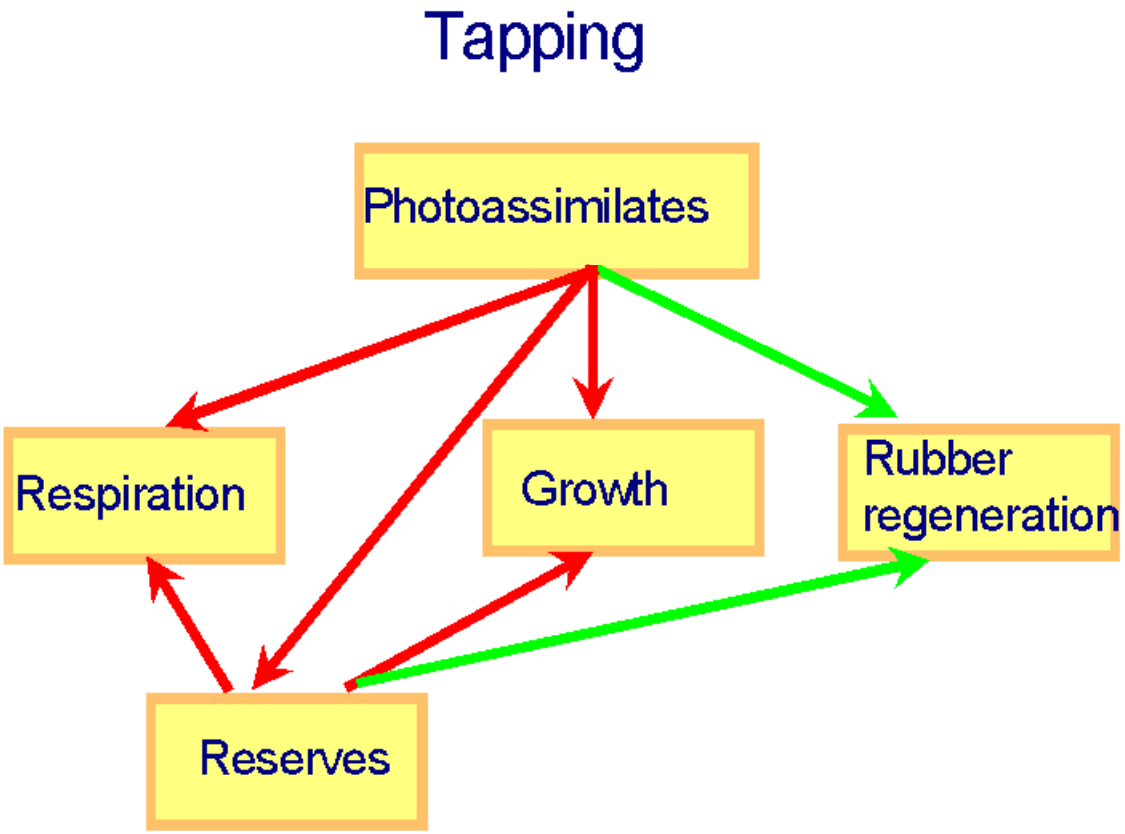


Tapping and latex harvesting

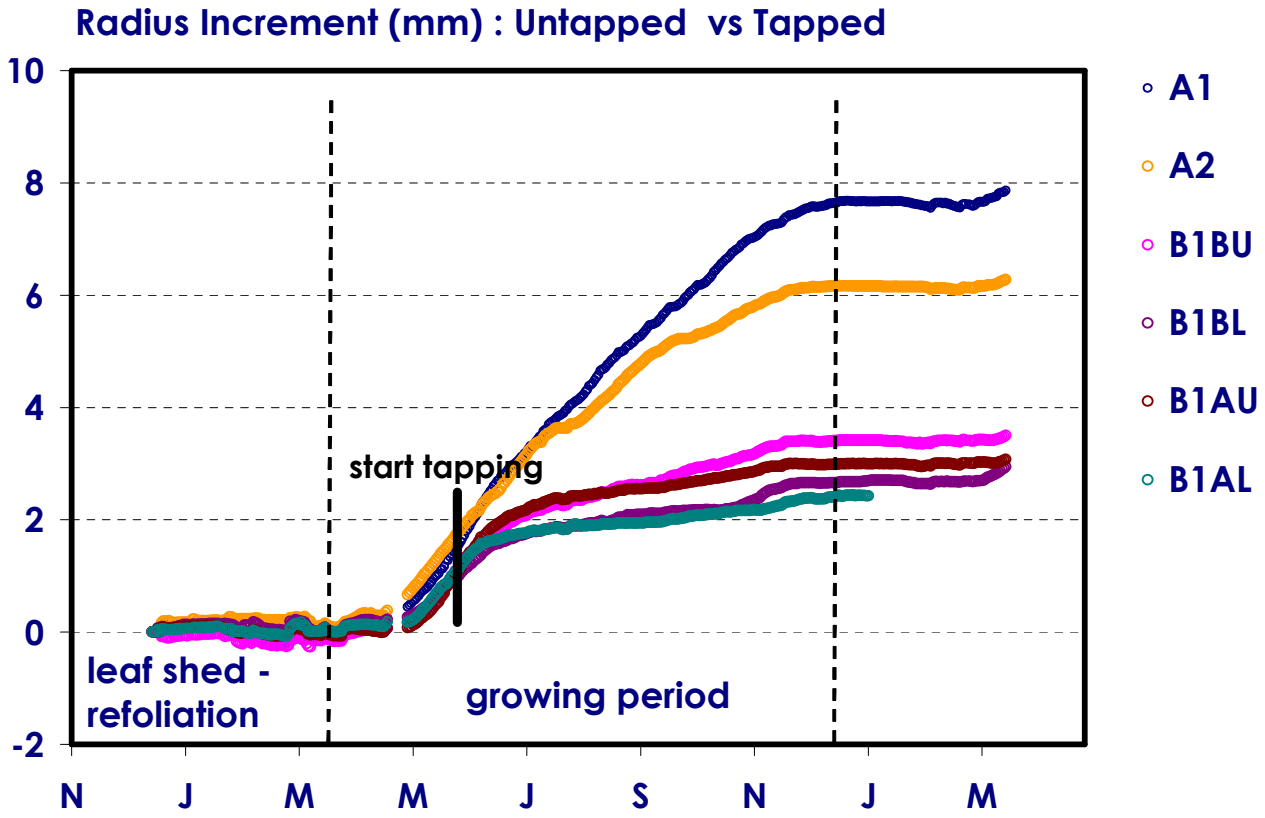
Tapping is local but its effect is extensive

- Major changes in whole trunk metabolism and functions

- According to most parameters, untapped panel is very different from untapped tree

Tapping and radial growth

The quick decrease in radial growth following tapping is really striking



U SILPI, P THALER, P KASEMSAP, A LACOINTE, A CHANTUMA, B ADAM, E GOHET, S THANISAWANYANGKURA and T AMÉGLIO Tree Physiol 2006

Tapping and radial growth

- **Direct competition for assimilate enough to explain such dynamics of the reserves ?**

- **Maybe a direct effect of tapping itself (wounding, stress hormonal signal) ?**

Tapping and latex harvesting

First characteristic : Modification of natural metabolism

- Tapping diverts photoassimilates to a new sink : the latex regeneration sink.This decreases the amount of assimilates available for growth and reserves
- The more the tree produces, the more its growth is reduced : A high precocious yield will reduce the future yields (Competition between short run yield and long run yield)
- The balance between radial growth and vertical growth is modified (increased susceptibity to wind damage)

Tapping and latex harvesting

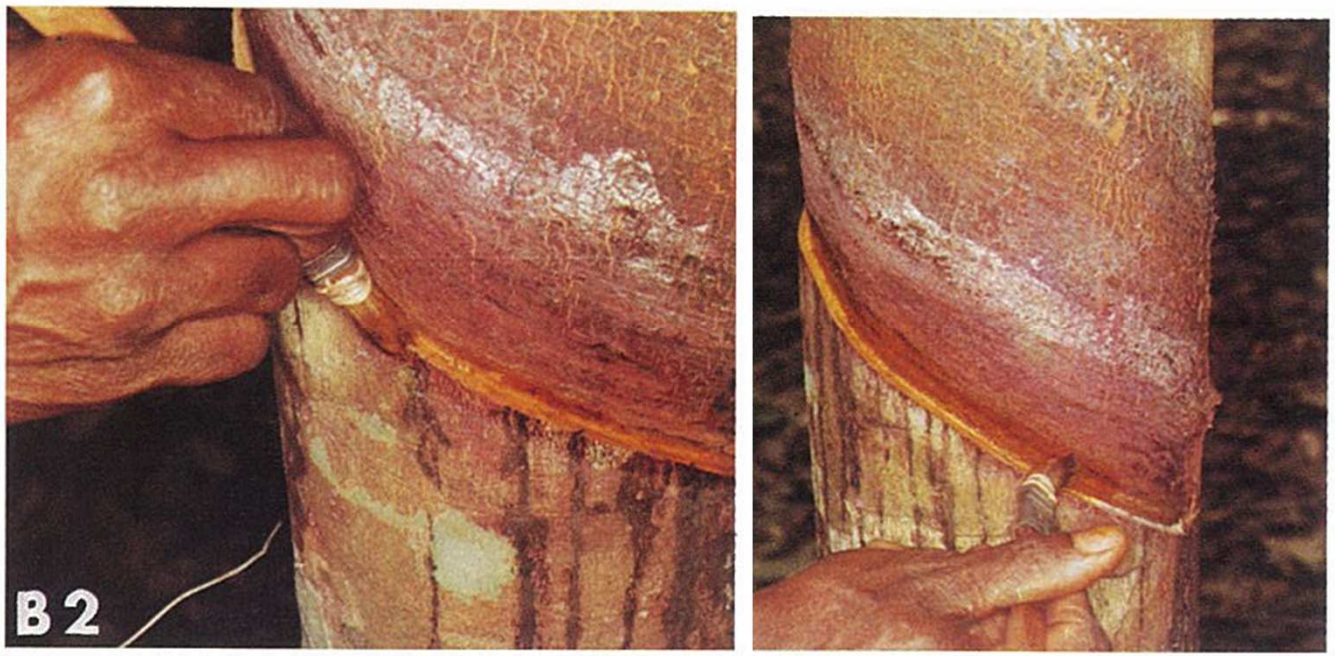
Second characteristic : Against nature

- The latex is a medium organized to coagulate as soon as it is in contact with the outside (lutoid/hevein/acetylglucosamine system)
- It is now thought that the latex is a defense system, which allows, after coagulation, a good protection and healing of wounds (like animal blood)
- Moreover, antibiotic and antifungic molecules are present inside the latex : glucanases, chitinases...
- In the contrary, the goal of the rubber planter is to obtain a long latex flow, in order to increase his rubber yield and his income.

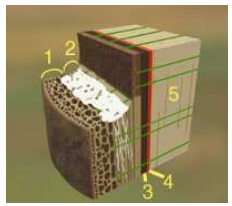
Latex metabolism latex harvesting: tapping and stimulation

Intensifying tapping system:

- High tapping frequency (d/2; 2d/3; 3d/4)
- Application of ethylene stimulant on the tapping cut



Latex cell metabolism latex harvesting: tapping and hormonal stimulation



Stimulating treatments of Latex yield

WOUNDING
- The normal tapping
- scrapping of bark

HORMONAL TREATMENTS
- Auxine: IAA
- 2,4-D, 2,4,5-T
- NAA, MCPA ...

CHEMICALS TREATMENTS
- $CuSO_4$
- H_3BO_3
- Hg_2Cl_2
- H_2O_2

ACETYLENE
Ethylene analogous

ETHREL
Ethylene releaser

Ethylene
The key stress plant hormone

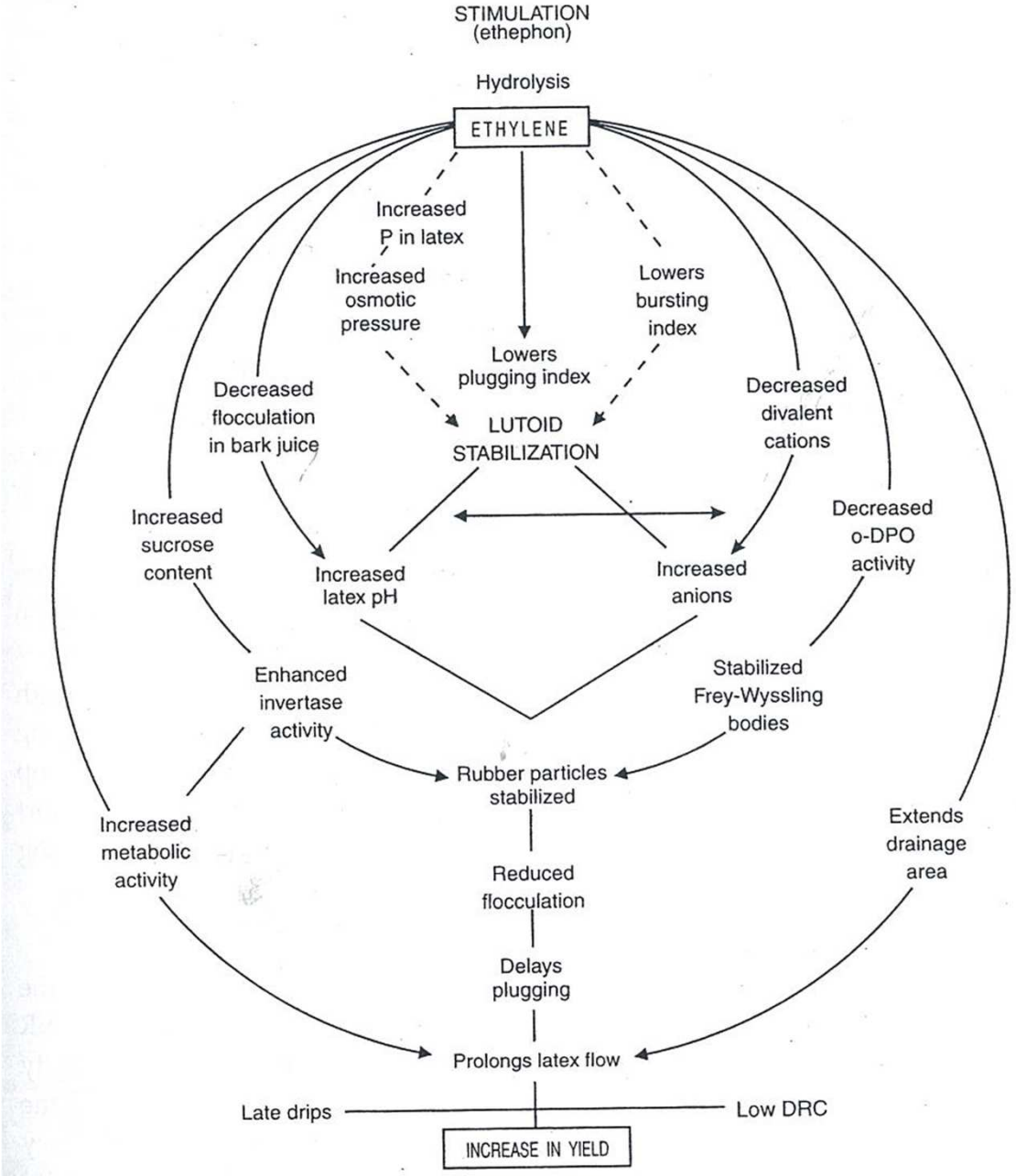
C2H4

ENDOGENOUS PRODUCTION OR EXOGENOUS

- INCREASE IN DURATION OF LATEX FLOW
- INCREASE OF SINK EFFECT AND OF LATEX METABOLISM ACTIVITY

INCREASE OF LATEX YIELD

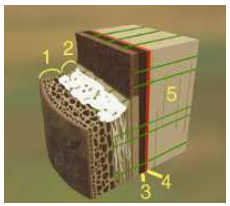
Flow chart of the concepts of ethylene action in latex cell metabolism and yield

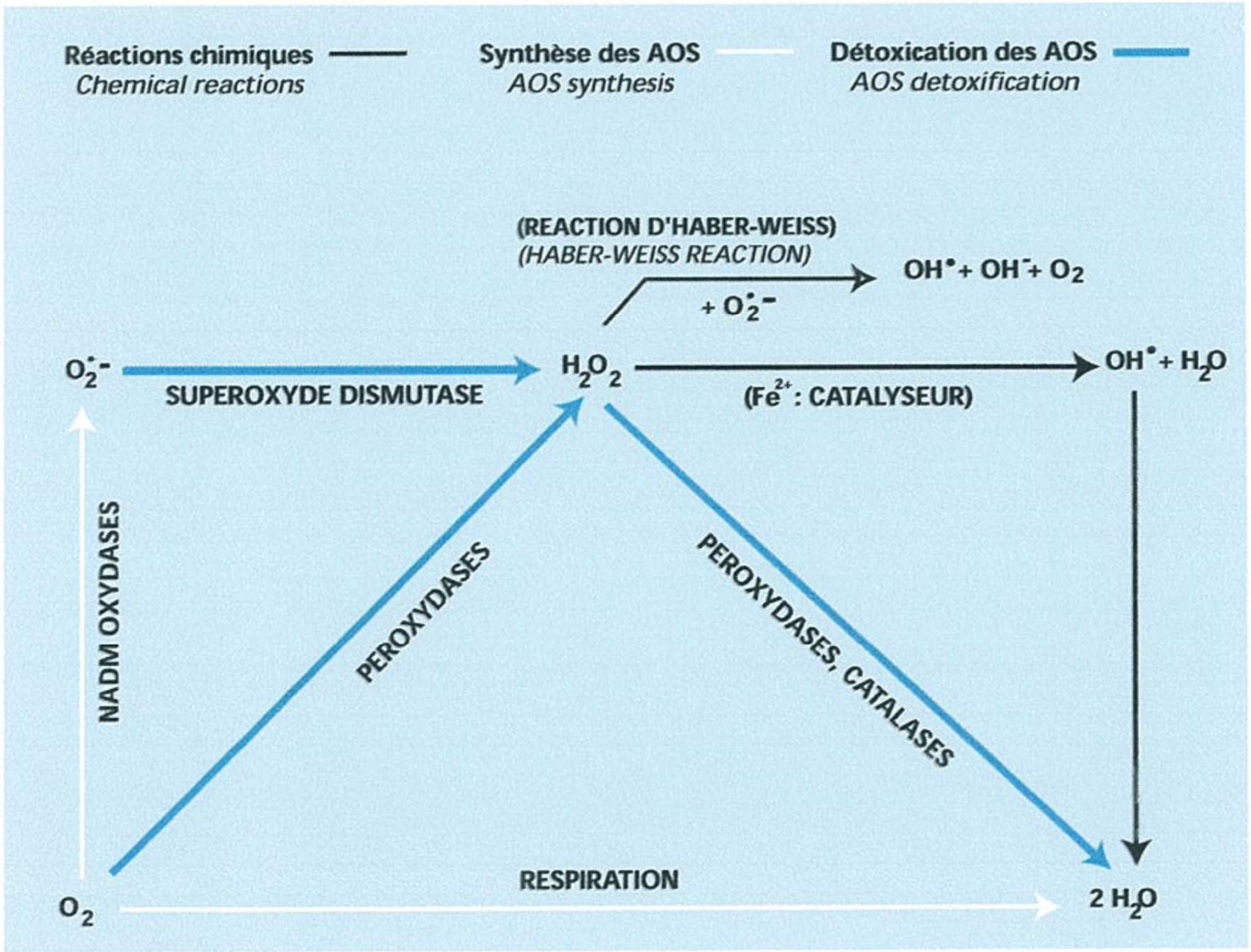


Particularities of the rubber yield

Metabolism of the latex cell: senescence mechanisms and defence,

Active Oxygen Species (AOS): production and detoxication

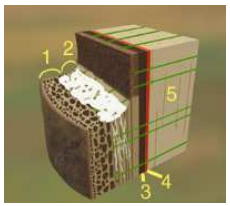


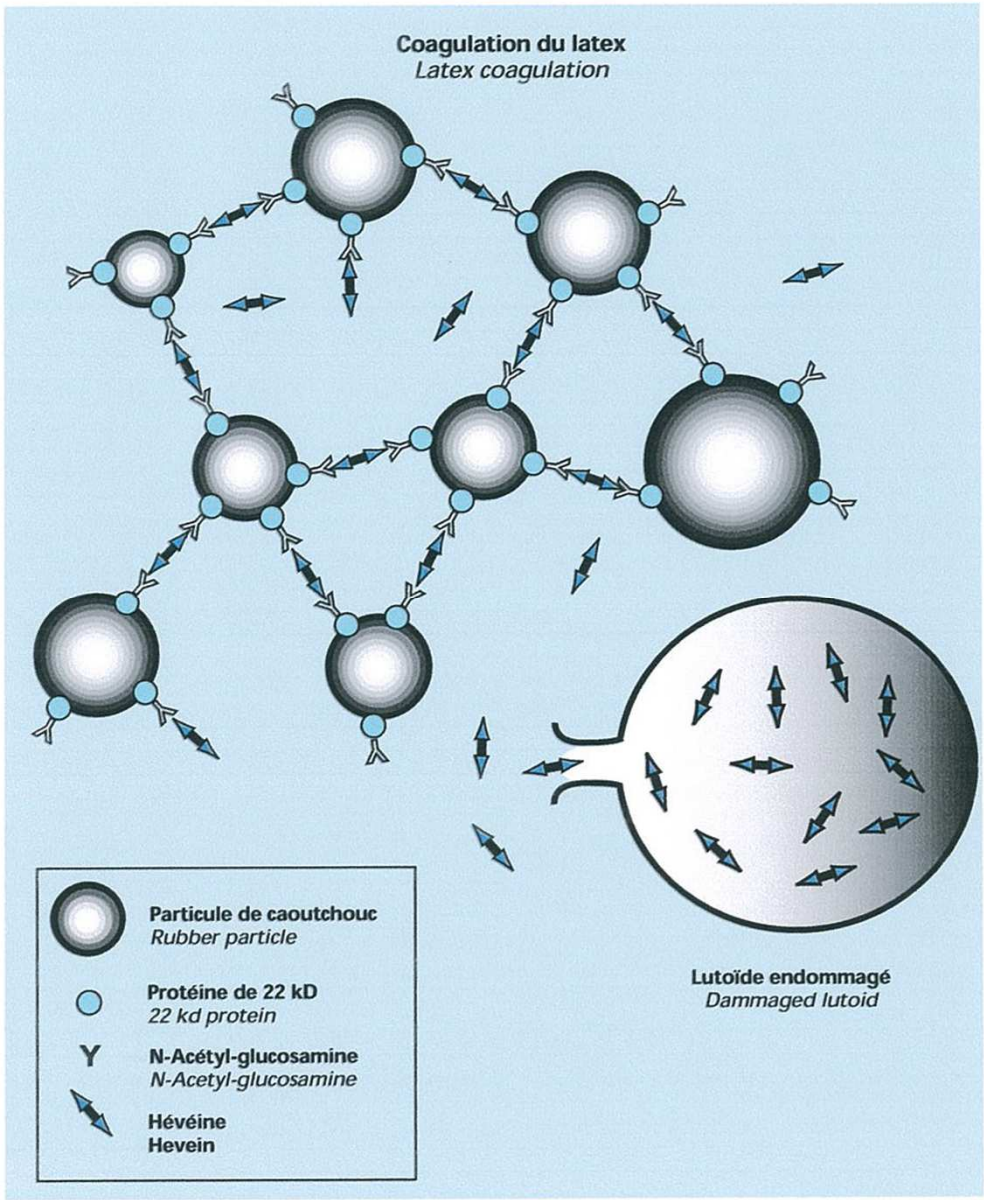


Jacob J.L, Prévôt J.C., Lacote R., Gohet E., Clément A., Gallois R.1, Joet T.1, Pujade-Renaud V.2, d'Auzac J. 1988

Particularities of the rubber yield

Metabolism of the latex cell: latex coagulation, lectin hypothesis



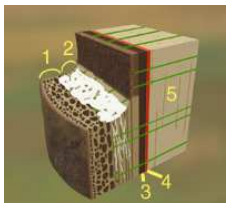


Jacob J.L, Prévôt J.C., Lacote R., Gohet E., Clément A., Gallois R.1, Joet T.1, Pujade-Renaud V.2, d'Auzac J. 1988

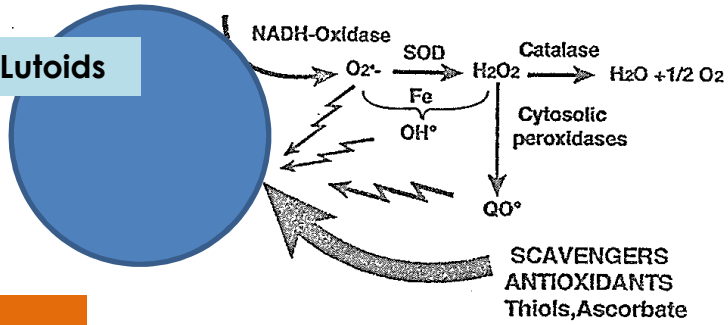
The lutoids
The key organite
in latex flow

Particularities of the rubber yield

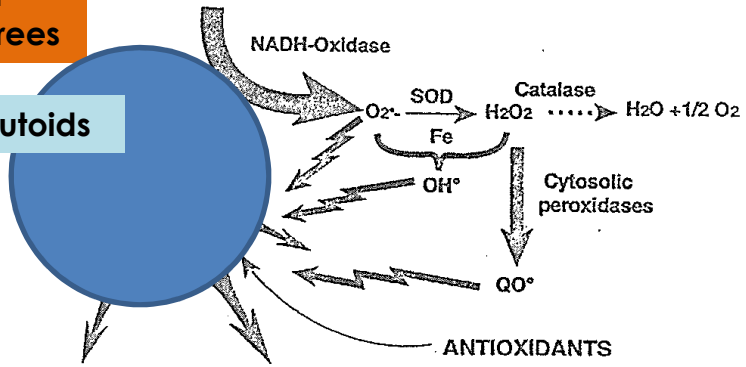
Metabolism of the latex cell and rubber biosynthesis: homeostasis



High Yielding and normal trees



Over-exploited and dry trees

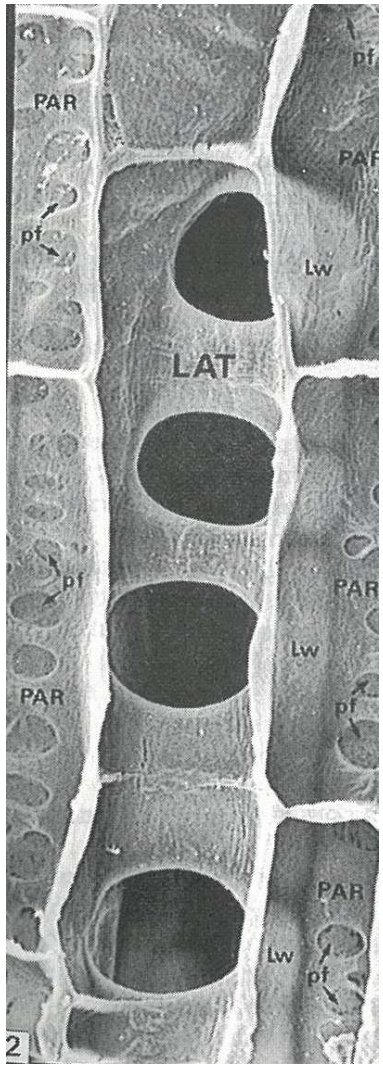


Dysfunctionment of latex metabolism and plugging

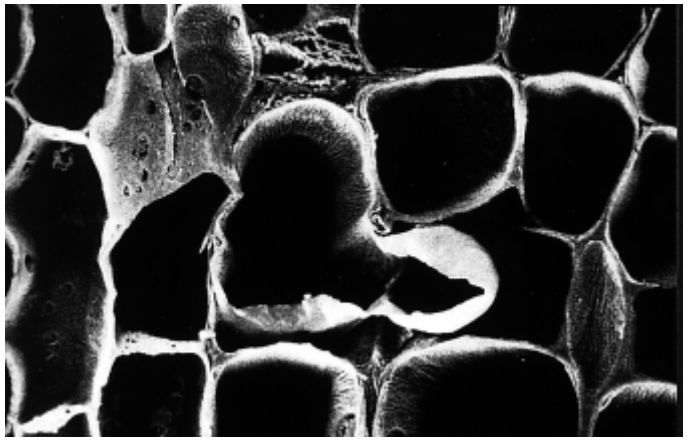
oxidative degradation of the lutoid membrane and Tapping Pannel Dryness

Chrestin H. 1984

The risk of over-stimulation: decompartimentalization and thrombosis and tapping panel dryness



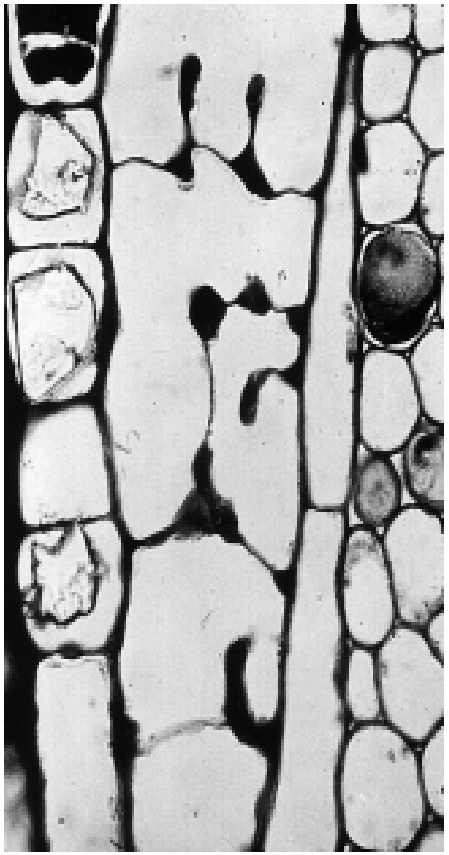
Longitudinal radial view of an healthy laticifer



Tylosoid formation: a parenchyma cell invading the laticifer cell on its right (scanning microscopy, Faÿ-Hébant).

Hebant et de Fay 1981

De Fay, Sanier et Hébant 1988



Longitudinal section of a laticifer invaved by a cluster of cells formed from an anarchically multiplied tylosoid (transmission electron microscopy, Faÿ-Hébant).

The risk of over-stimulation: lutoids membranes deterioration

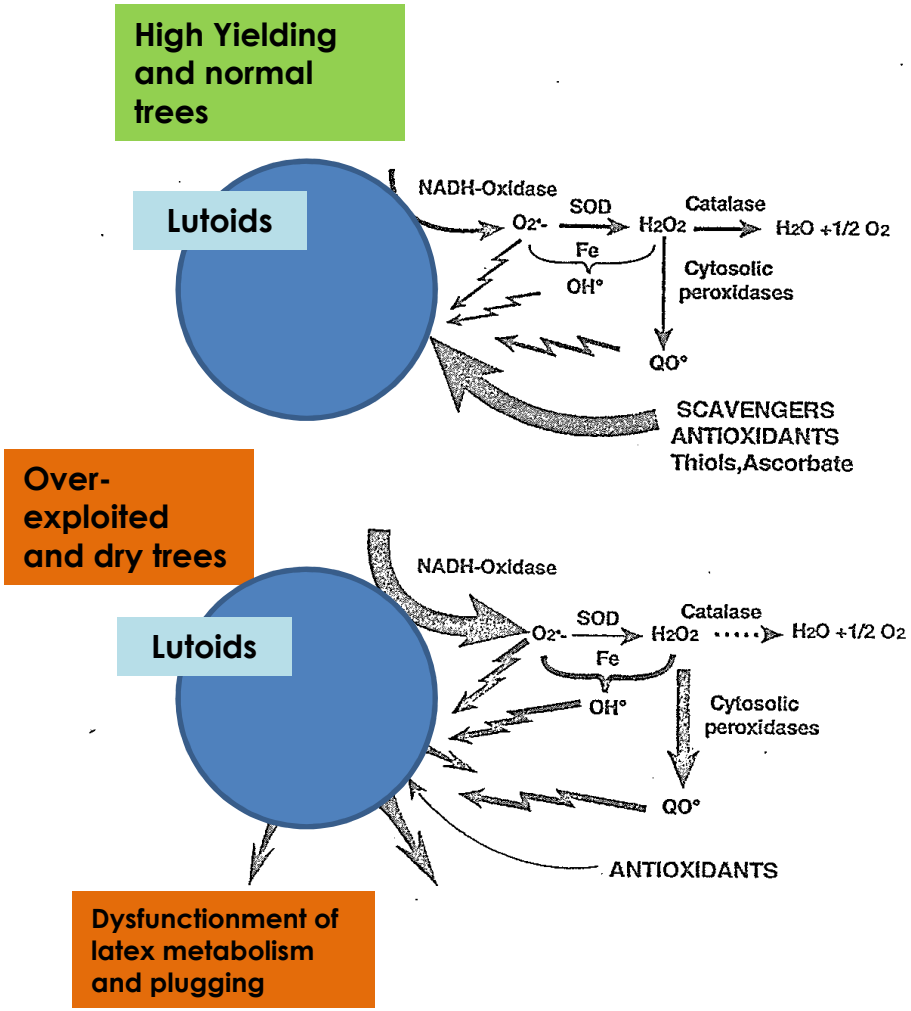


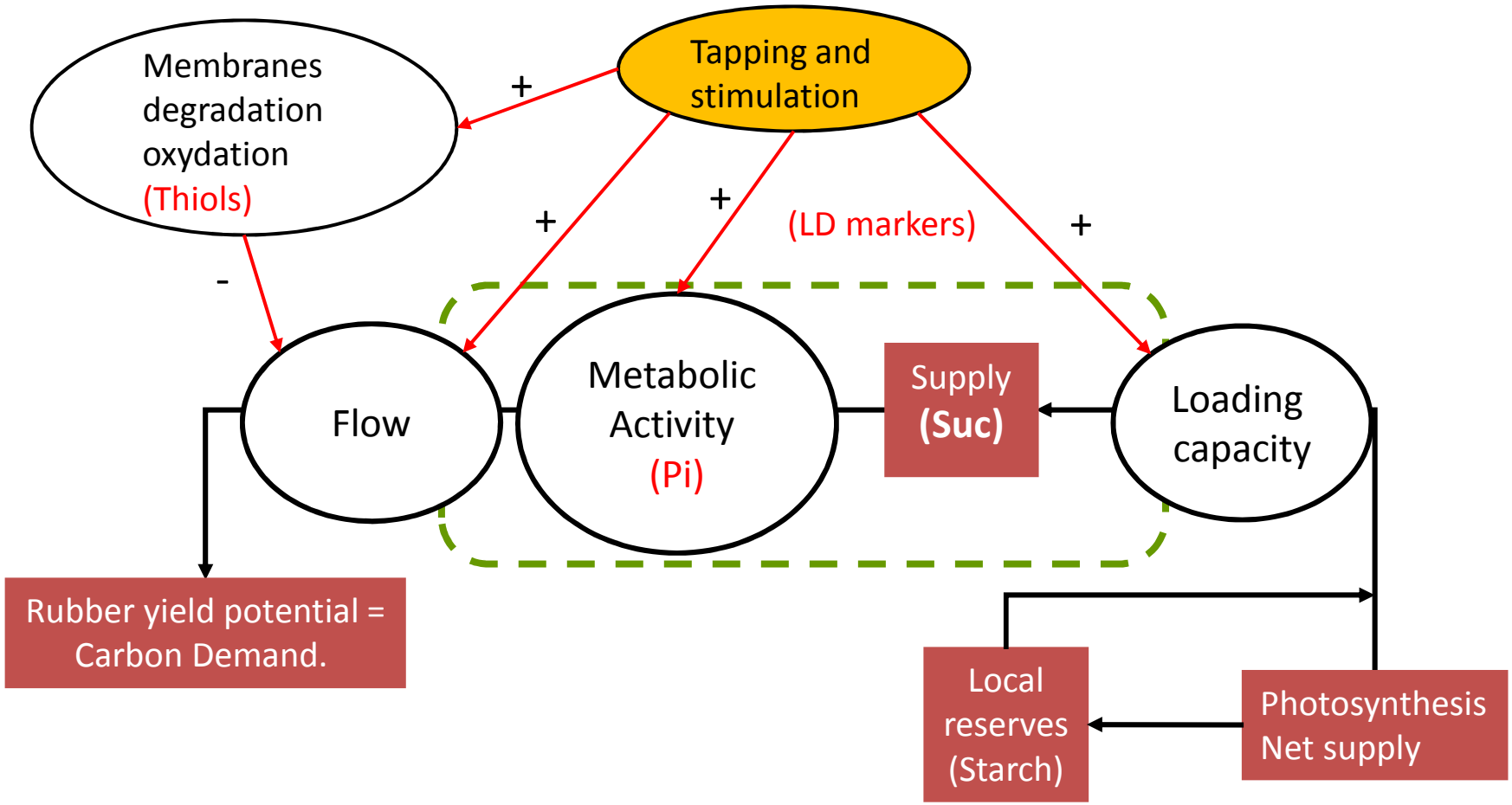
Figure 23 : Peroxidative degradation of the lutoid membrane and Tapping Pannel Dryness

Chrestin 1984

Tapping panel dryness

The brownbast: bark necrosis

Latex cell metabolism latex harvesting



Metabolic demand, estimated by Pi, determines, together with flow, the C demand.

C supply depends on sucrose uploading capacity.

Rubber tree productivity: latex harvesting and Latex Diagnostic

A. Many possible yields of rubber tree :
Optimization of tapping systems in order to obtain a physiological yield (short run and long run yield)

B. Against the natural process :
(Early coagulation in order to heal wounds)

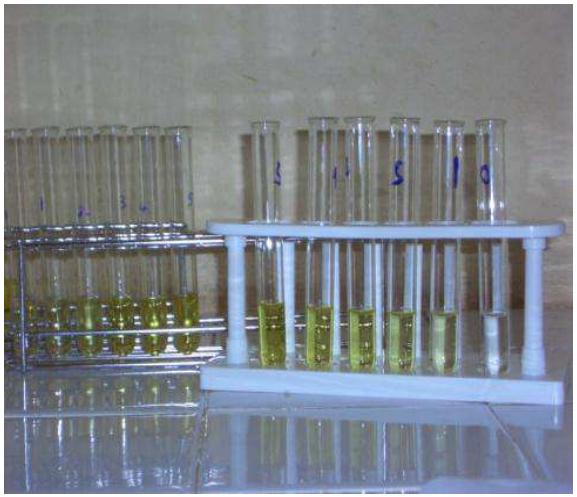
C. Modification of the natural metabolism and architecture of the tree

NEED OF A PHYSIOLOGICAL DIAGNOSIS TO :

- OPTIMIZE TAPPING SYSTEMS
- ASSESS THE CLONAL YIELD POTENTIAL

Rubber tree productivity: latex harvesting and Latex Diagnostic

The latex diagnosis : DRC, SUC, Pi, RSH



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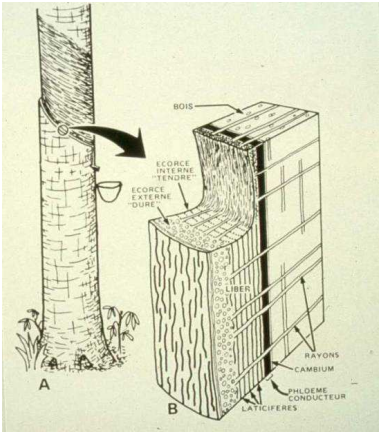
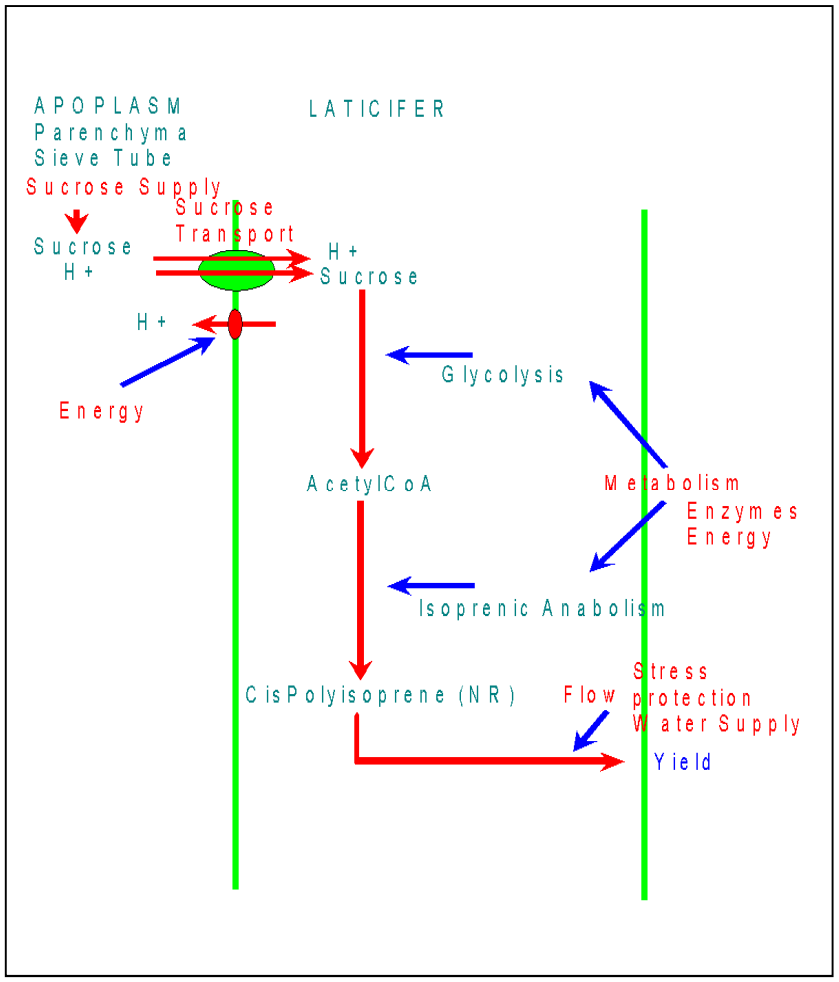
Latex cell metabolism





DRC

Sucrose

Pi

R-SH

Latex diagnosis

- Characterization of the metabolism
- Clones typology

Significance of Sucrose

- **Regeneration :**

Sucrose is the molecule which initiatesisoprenic synthesis.
(Lynen 1969)

A low sucrose content may indicate
* an inadequate hydrocarbon supply
* an active utilisation of the sugar (active metabolism)

A high sucrose content may indicate
* a very active supply (clonal characteristic)
* a defective utilisation of the sugar (inactive metabolism)

Sucrose is the potential energy of the latex cell. It represents a major component of the clonal yield potential (Lacote 1991, Gohet 1996)

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Significance of inorganic (or mineral) Phosporus (Pi)

- Regeneration :

Mineral phosphorus (Pi) is tightly associated with the energy metabolism of the latex cell :

* ATP turnover
* Enzymes phosphorylation
* Reducing cofactors (NADPH)
* Rubber chain elongation (production of PPi by rubber transferase, PPi hydrolysis)

High positive correlation with total rubber production (g/t/y)

Significance of Thiols (R-SH)

- **Flow :**

  Status of the antioxydant systems and subcellular membrane protection processes

  Low thiols indicate that the in situ peroxidation processes are important and not sufficiently inhibited.
  This can lead to destabilization of organelles and in particular of lutoids (bursting), resulting in faster coagulation. (Chrestin 1984, 1985)

- **Regeneration :**

  Activators of key enzymes in the metabolism : invertase, pyruvate kinase. (Jacob and al, 1981, 1982)

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Significance of TSC/DRC

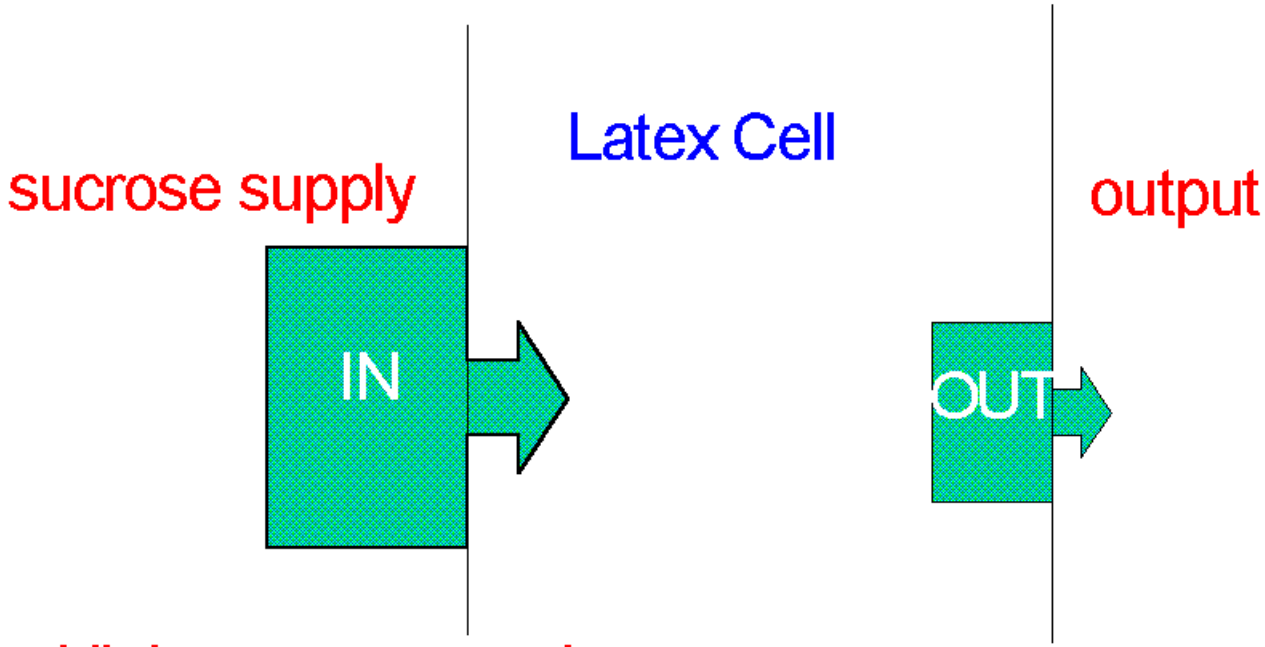
- **Flow :**

  High TSC or DRC values increase the latex viscosity and can slow down the latex flow.
  (Brzozowska-Hanover *and al.*1979)

- **Regeneration :**

  TSC or DRC reflect in situ metabolic activity.
  Too low values indicate inadequate reconstitution of cells materials (rubber and other components) between two tappings.
  (Eschbach *and al.*1984, Prevot *and al.*1984)

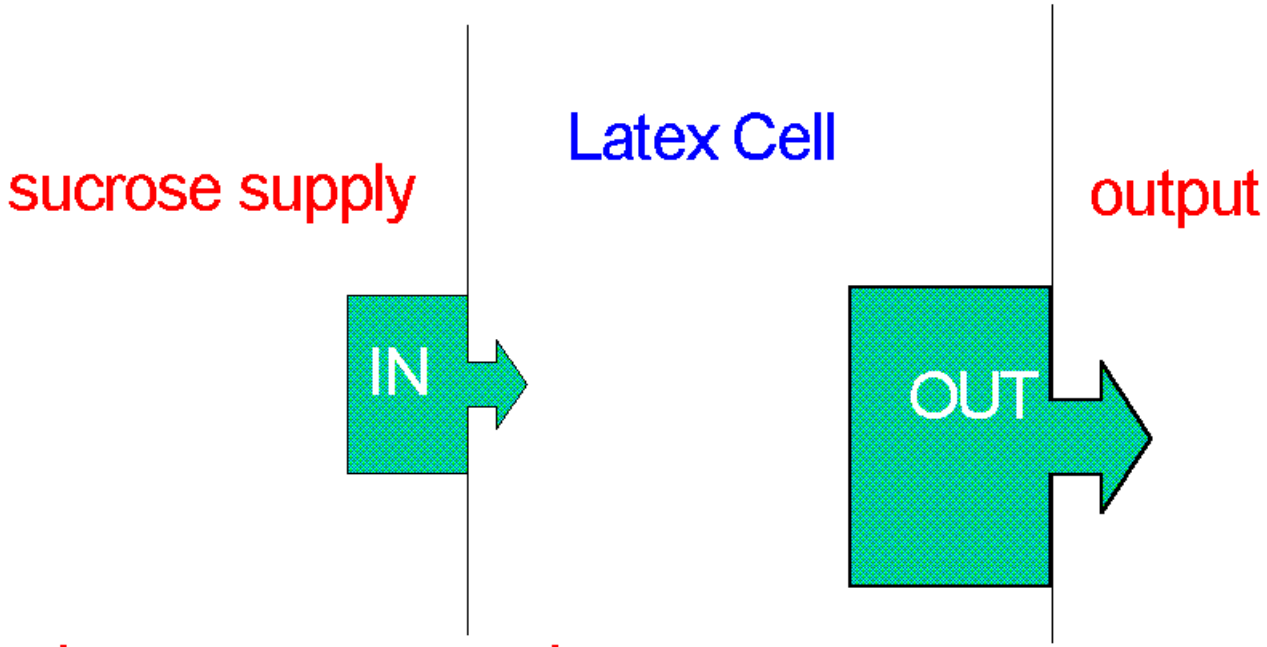
LATEX DIAGNOSIS : INTERPRETATION KEYS



High sucrose supply
Low metabolic demand / low output
Diagnosis of Under Exploitation

TSC DRC	- / = / +	Pi x RSH low
Suc	+	
Pi	-	
RSH	- / = / +	

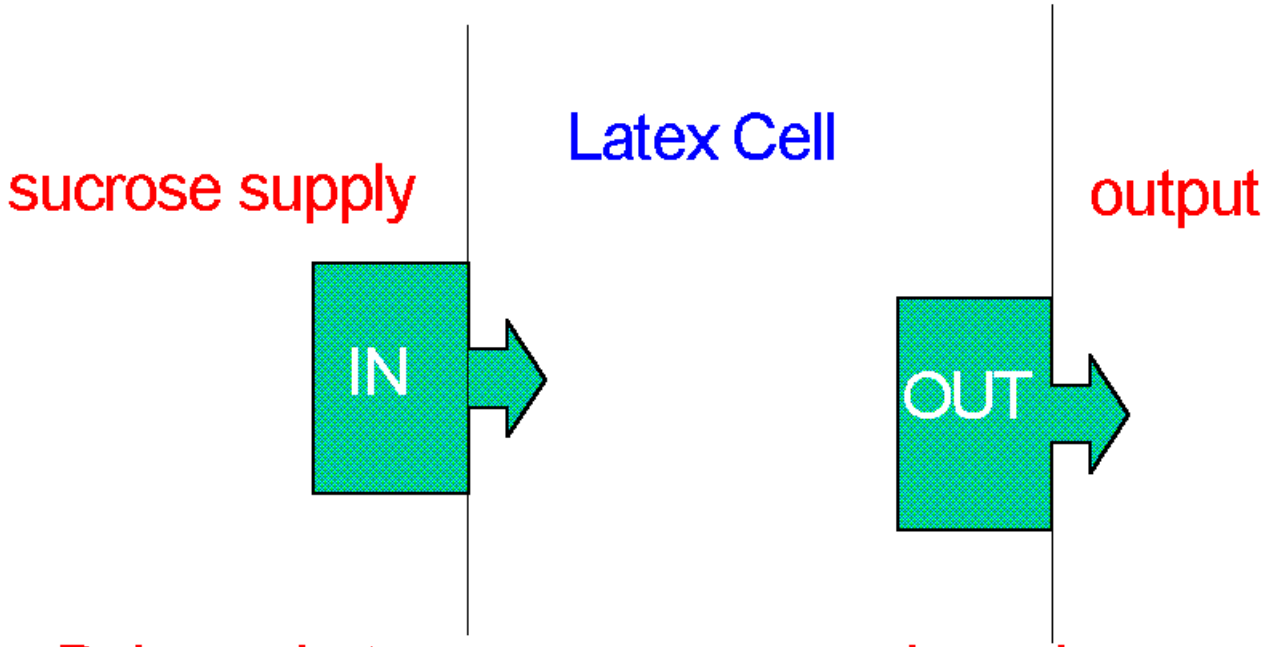
LATEX DIAGNOSIS : INTERPRETATION KEYS



Low sucrose supply
High metabolic demand / High output
Diagnosis of Over Exploitation

TSC DRC	- / =	Pi x RSH low
Suc	-	
Pi	+ / = / -	
RSH	-	

LATEX DIAGNOSIS : INTERPRETATION KEYS



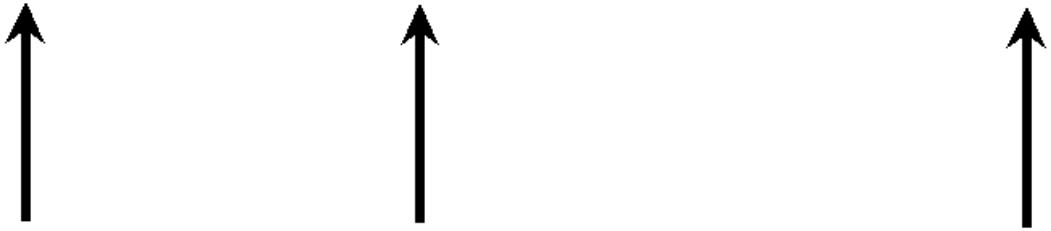
Balance between sucrose supply and metabolic demand

Diagnosis of Balanced Exploitation

TSC DRC	=	Pi x RSH max
Suc	=	
Pi	+	
RSH	=	

Clonal typology of the latex functioning and potential yield

$$P_{MAX} = P_0 + a\ dSuc$$



Yield potential	Yield without stimulation	Available Sucrose
(a # 0,06 in Ivory Coast)	Metabolism yield component	Reserve yield component : Possibility of Intensification

$Po = f(metabolism)$

$dSuc = f(SucPo, dPi)$

Where does the sucrose come from?
Are reserves mobilized?

cirad

Physiological modeling :

Latex Clone Typology

- Latex clone typology is based on combination of :
  - Latex metabolism :
    - Speed of latex regeneration
    - Initial level of production before stimulation
  - Latex sugar loading :
    - Sugar reserve available for intensification (response and tolerance to stimulation, tolerance to stress)

Physiological modeling : Latex Clone Typology

- Latex clone typology :
  - Latex metabolism can be described by 5 classes (low, low-medium, medium, medium-high and high)
  - Latex sugar loading can be described by 3 classes (low, medium and high)

⇒ MATRIX [5,3] : 15 possible types of clones

⇒ MODELING

Gohet et al 2011

Physiological modeling : Stimulation and Yield Potential

	Low Metabolism Met-	Low-Medium Metabolism Met -=	Medium Metabolism Met =	Medium-High Metabolism Met =+	High Metabolism Met +
Low Sugar Loading (Suc -)	***Typology c1*** ***Met - Suc -*** AVROS 2037	***Typology c3*** ***Met -= Suc -*** MEDIUM STIM	***Typology c6*** ***Met = Suc -*** LOW STIM	***Typology c9*** ***Met =+ Suc -*** VERY LOW STIM	***Low probability***
Medium Sugar Loading (Suc =)	***Typology c2*** ***Met - Suc =*** AF 261 VERY HIGH STIM	***Typology c4*** ***Met -= Suc =*** PB 86 PR 107 HIGH STIM	***Typology c7*** ***Met = Suc =*** GT1 PB 254 RRIC 100	***Typology c10*** ***Met =+ Suc =*** RRIM 600 BPM 1 BPM 24 RRIC 110 PR 255 PB 5/51 IRCA18 IRCA 109 PB 330	***Typology c12*** ***Met + Suc =*** PB 235 PB 260 PB 340 RRIM 901 RRIM 911 PR 261 IRCA 111 IRCA 130 IRCA 209
High Sugar Loading (Suc +)	***Low probability***	***Typology c5*** ***Met -= Suc +*** PB 217	***Typology c8*** ***Met = Suc +*** RRIC 121	***Typology c11*** ***Met =+ Suc +*** IRCA 19 IRCA 41 RRIM 921	***Typology c13*** ***Met + Suc +*** IRCA 230 RRIM 712

STIMULATION INTENSITY MUST BE DECREASED FROM LEFT (Low metabolism) TO RIGHT (High metaboilism)
STIMULATION INTENSITY MUST BE INCREASED FROM TOP (Low sucrose loading) TO BOTTOM (High sucrose loading)
IN COLOUR : HIGH YIELD POTENTIAL
North West -South East DIAGONALS : HOMOGENOUS STIMULATION RECOMMENDATIONS (5)

Rubber tree productivity and sustainability

Preserving the latex cells functioning

Tapping systems :
frequency
cut length
panel management

Physiological equilibrium
LATEX DIAGNOSIS

Stimulation :
EthEphon
Ethylene gaz

DRC

Phloem assimilates
Hydrocarbon reserves

Regeneration
Oxydative stress

Flow /
Coagulation

Drainage area

Dryness cut

ECOPHYSIOLOGY of the PRODUCTION
Competition latex - biomass :
Circonference and growth
+ loss with respiration

Reduction
of girth

Focus on carbohydrate reserves

Facts

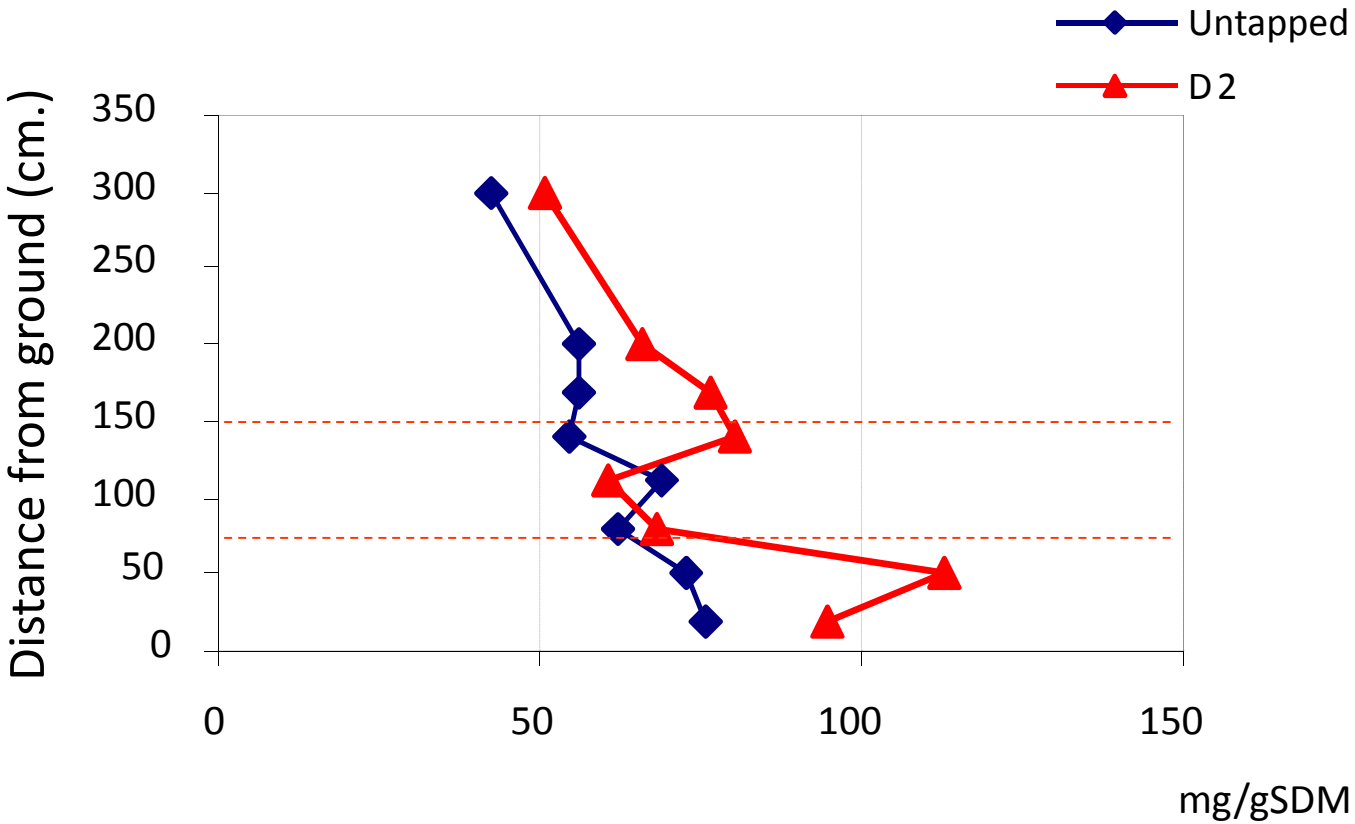
- Decrease of intra-laticiferous sucrose in intensively exploited trees.

- Some tapping systems or clones maintain a higher sucrose content (balance) than others.

Hypotheses

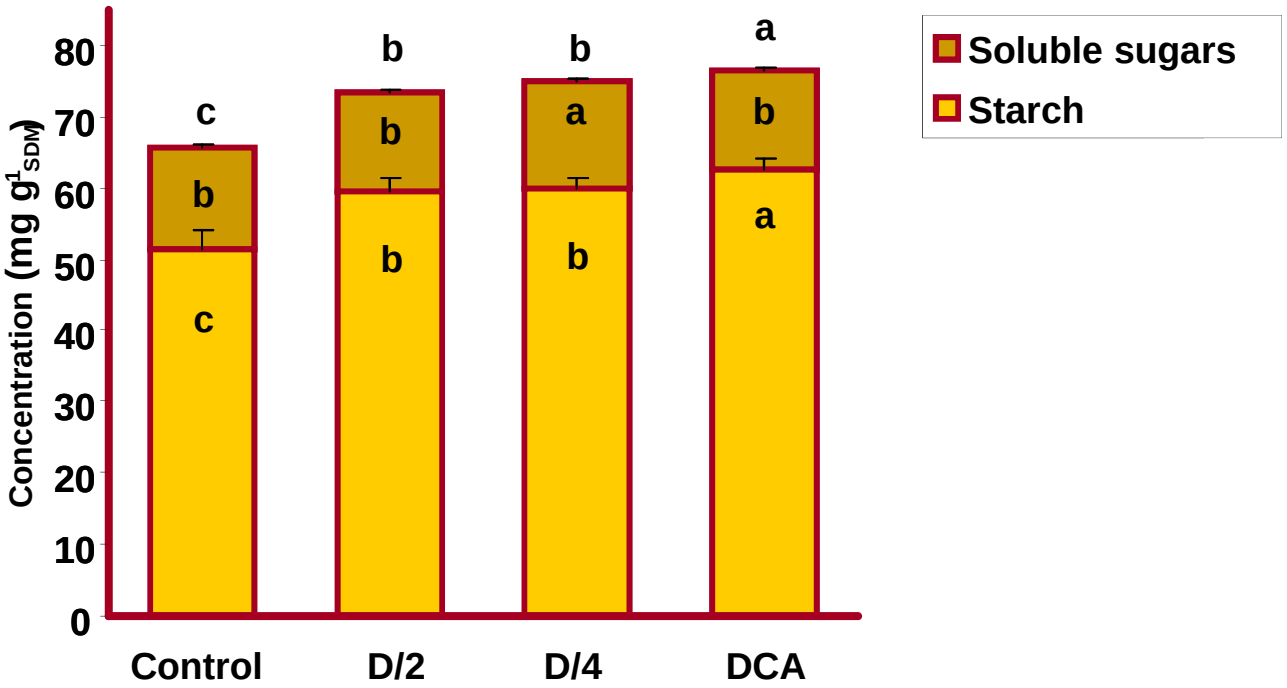
- Differences in the amount/activity of sucrose transports and/or differences in reserves availability within and around the drained area (tapping panel)

Vertical distribution starch in wood



Vertical gradients of starch deeply disturbed by tapping : decrease within the renewing bark area but increase below the tapping cut

Carbohydrate in wood



Starch is the main carbohydrate in wood.Higher Starch and TNC in tapped treatments than in control.

Higher Starch and TNC in DCA than in other tapped trees.

P. CHANTUMA, A. LACOINTE, P. KASEMSAP, S. THANISAWANYANGKURA, E. GOHET, A. CLE MENT, A. GUILLIOT, T. AME´GLIO and P. THALER 2009 Tree Physiology 29, 1021–1031

Reserves dynamics and tapping/latex harvesting

- When an additional demand for carbon is created by tapping, wood reserves were not depleted but increased.
- Reserves are not a simple passive buffer. An increase in reserves can be prior to growth which is largely decreased following tapping.
- The trees seem to adapt the reserve level to the actual metabolic demand and to store carbohydrates to face the risk induced by tapping.

ARE CARBOHYDRATE RESERVES A RELEVANT PARAMETER TO ASSESS LONG TERM PERFORMANCE OF TAPPING SYSTEM ?

Latex regeneration

Energy requirement.

Active transport of sucrose across laticifer membrane.

Reserves

Mainly starch in wood and sucrose in bark.

are increased by tapping and hormonal stimulation. ►► **Rubber productivity**

Enzyme activities

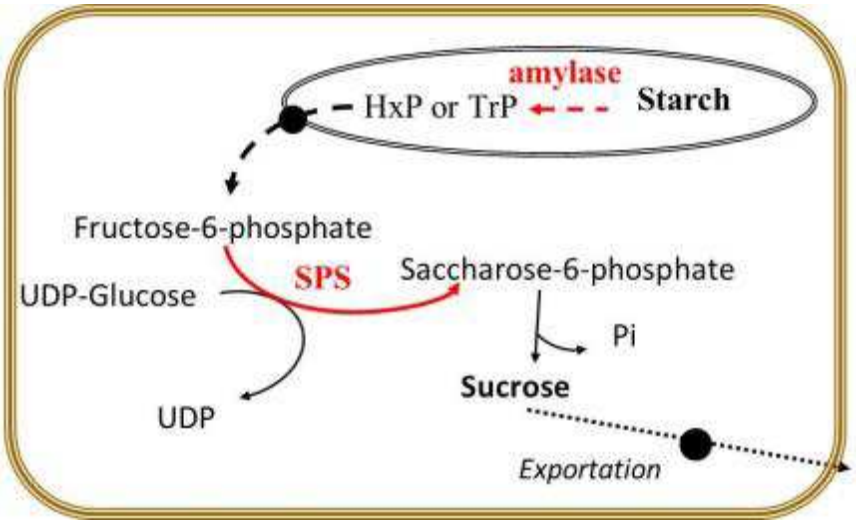
Enzymes control starch synthesis and hydrolysis as well as sucrose synthesis. ◄

►► Study of the enzymes involved in reserve formation and use in rubber tree

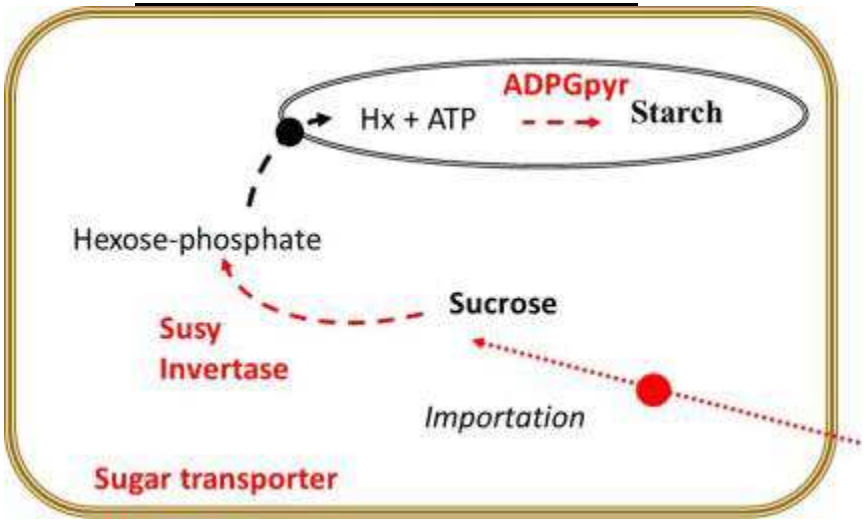
Prospects: Study of the enzymes involved in reserve formation and use in rubber tree, link to yield

Wood or bark cells

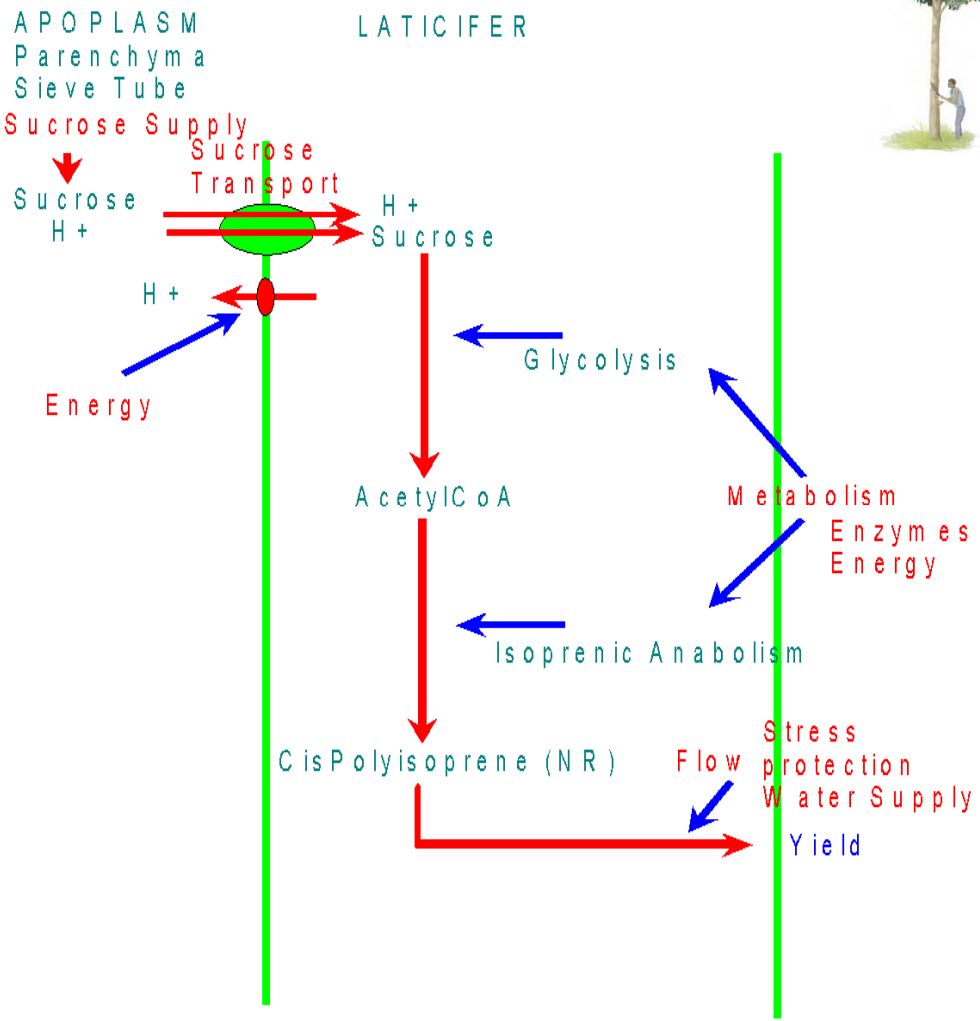
Study of the enzymes involved in reserve use in rubber tree



Study of the enzymes involved in reserve formation in rubber tree



Latex cells



eco&sols
HRPP
cirad
Khop Khun krap!
Merci de votre attention
BIOTEC
a member of NSTDA
cirad
INRA
SupAgro
Montpellier
IRD
Université du Maine