

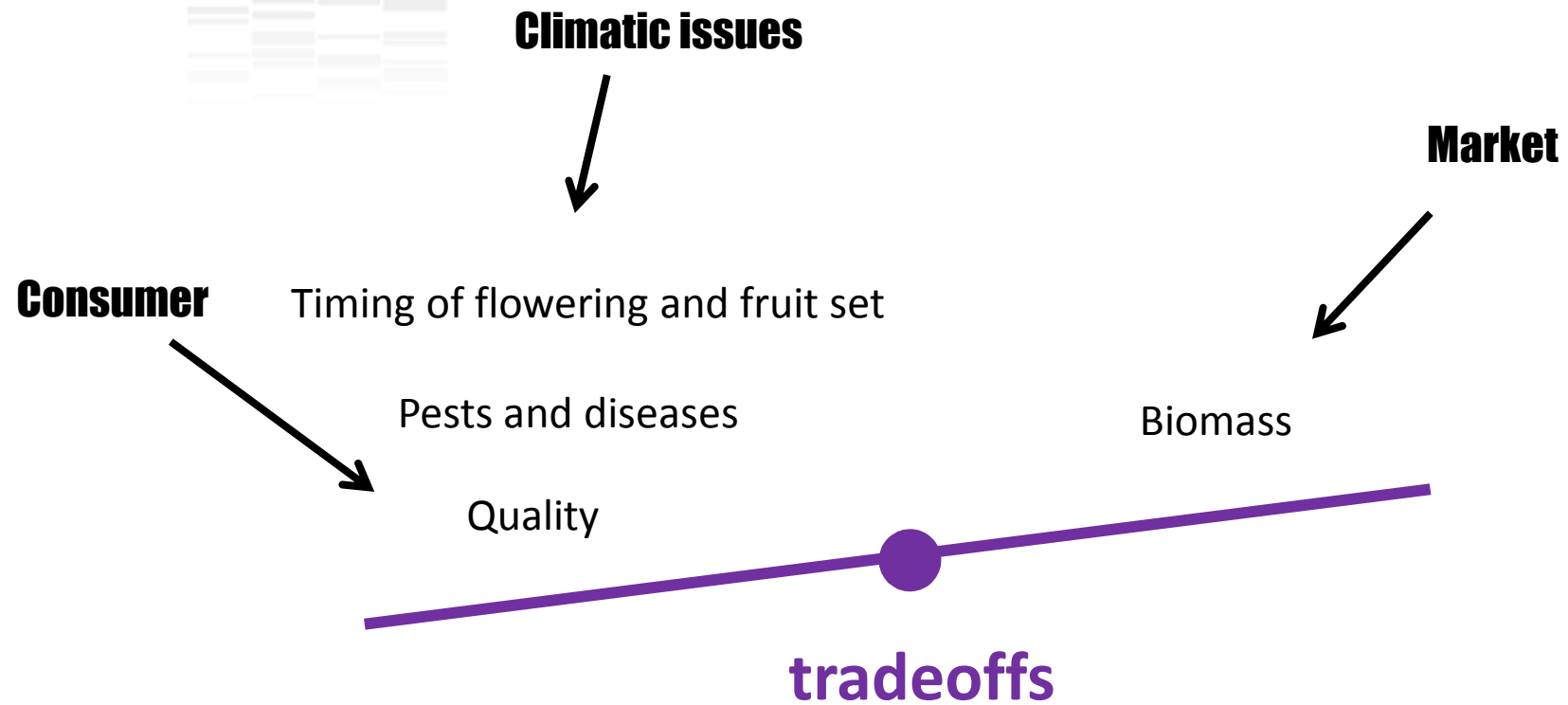


Mechanistic modelling of metabolism to understand and predict plant performance: a case study in growing tomatoes

Yves Gibon
Metabolism team
Fruit Biology & Pathology unit
INRA-Nouvelle Aquitaine & Bordeaux University



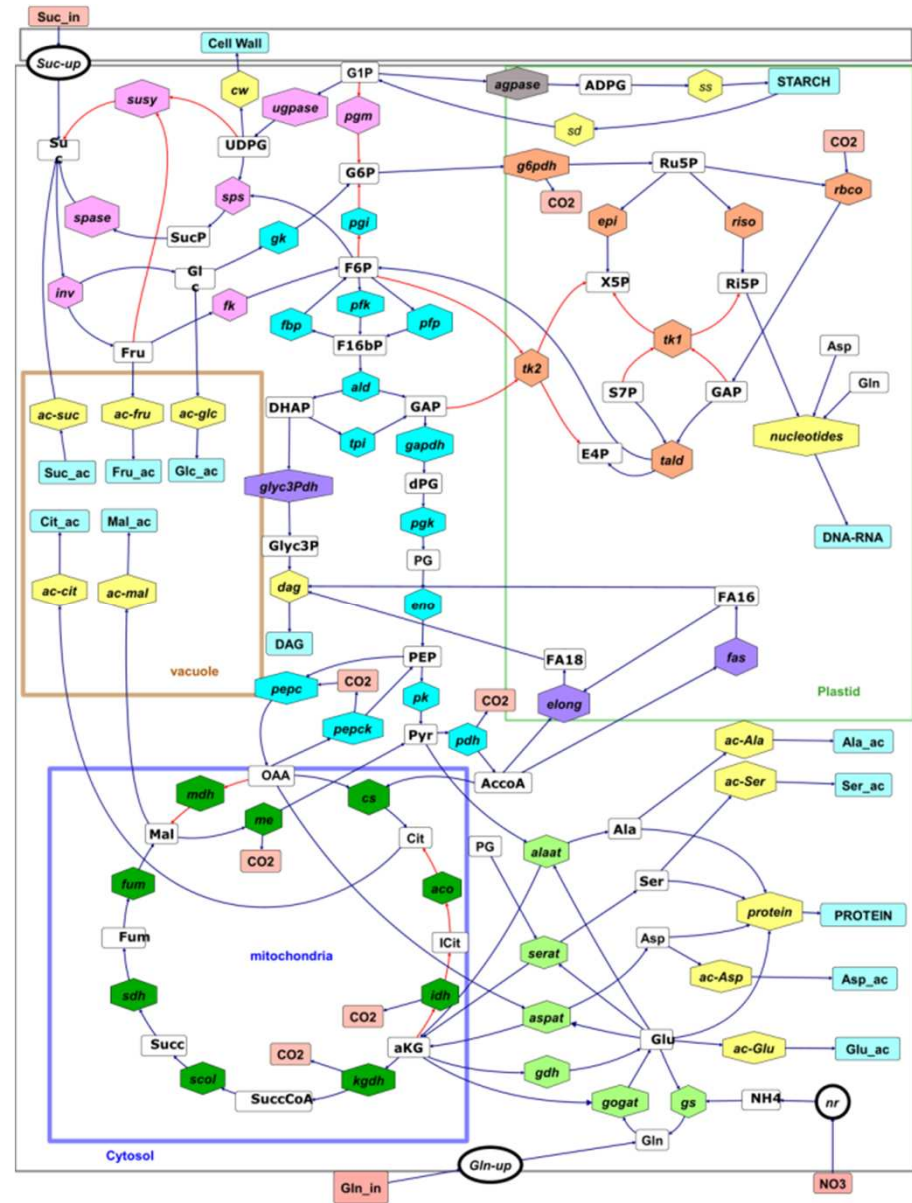
Topics at the Fruit Biology & Pathology unit



Plant metabolism

- The engine that provides building blocks and energy to growth
 - biomass production and quality
 - stress resistance
 - signalling

- Where are we?
 - topology getting well known
 - knowledge about individual steps improving
 - Very little known at the network level



Manipulating enzymes in order to enhance fruit production or quality?

Table III.

Role of carbohydrate genes in tomato fruit derived from transgenic manipulation.

Enzyme	Manipulation ¹	Fruit phenotype	Reference
Apoplastic invertase	<i>RNAi</i> ² , <i>CaMV</i> ³	↑sucrose, ↓ hexose, reduced fertility, fruit set and fruit size	Zanor <i>et al.</i> [25]
Acid invertase ⁴	Antisense ² , <i>CaMV</i>	↑ sucrose, ↓ hexoses and fruit size	Ohyama <i>et al.</i> [24]; Klann <i>et al.</i> [17]
Sucrose synthase 1 ⁵	Antisense, <i>CaMV</i>	↓ sucrose unloading at 7 days after anthesis, ↓ fruit set	D'Aoust <i>et al.</i> [101]
Sucrose synthase 1 ⁵	Antisense, <i>2A11</i> ⁶	No detectable change in starch or sucrose levels	Chengappa <i>et al.</i> [102]
Hexokinase 1	Ectopic <i>AtHK</i> ⁷ , <i>CaMV</i>	↓ fruit size, seed dry weight, starch content, total soluble solids at breaker stage and red ripe stage	Menu <i>et al.</i> [22]
Fructokinase 1	Antisense, <i>CaMV</i>	Delayed flowering, ↑ fruit sucrose	Odanaka <i>et al.</i> [23]
Fructokinase 2	Antisense, <i>CaMV</i>	↓ seed number, flower and fruit set, ↑ fruit sucrose	Odanaka <i>et al.</i> [23]
Vacuolar H ⁺ -ATPase	Antisense, <i>2A11</i>	↓ fruit weight, seed number, ↑ sucrose	Amemiya <i>et al.</i> [103]

¹ Transgenic manipulation and gene promoter used.

² *RNAi* and antisense methods are used to repress gene expression.

³ Cauliflower Mosaic Virus 35S gene promoter.

⁴ Both apoplastic and vacuolar acid invertase.

⁵ Isoform designation from Goren *et al.* [104].

⁶ Fruit-specific gene from tomato ("*2A11*") promoter.

⁷ Ectopic overexpression of *Arabidopsis* hexokinase 1.

Beckles *et al.* (2012) *Fruits* 67: 49–64

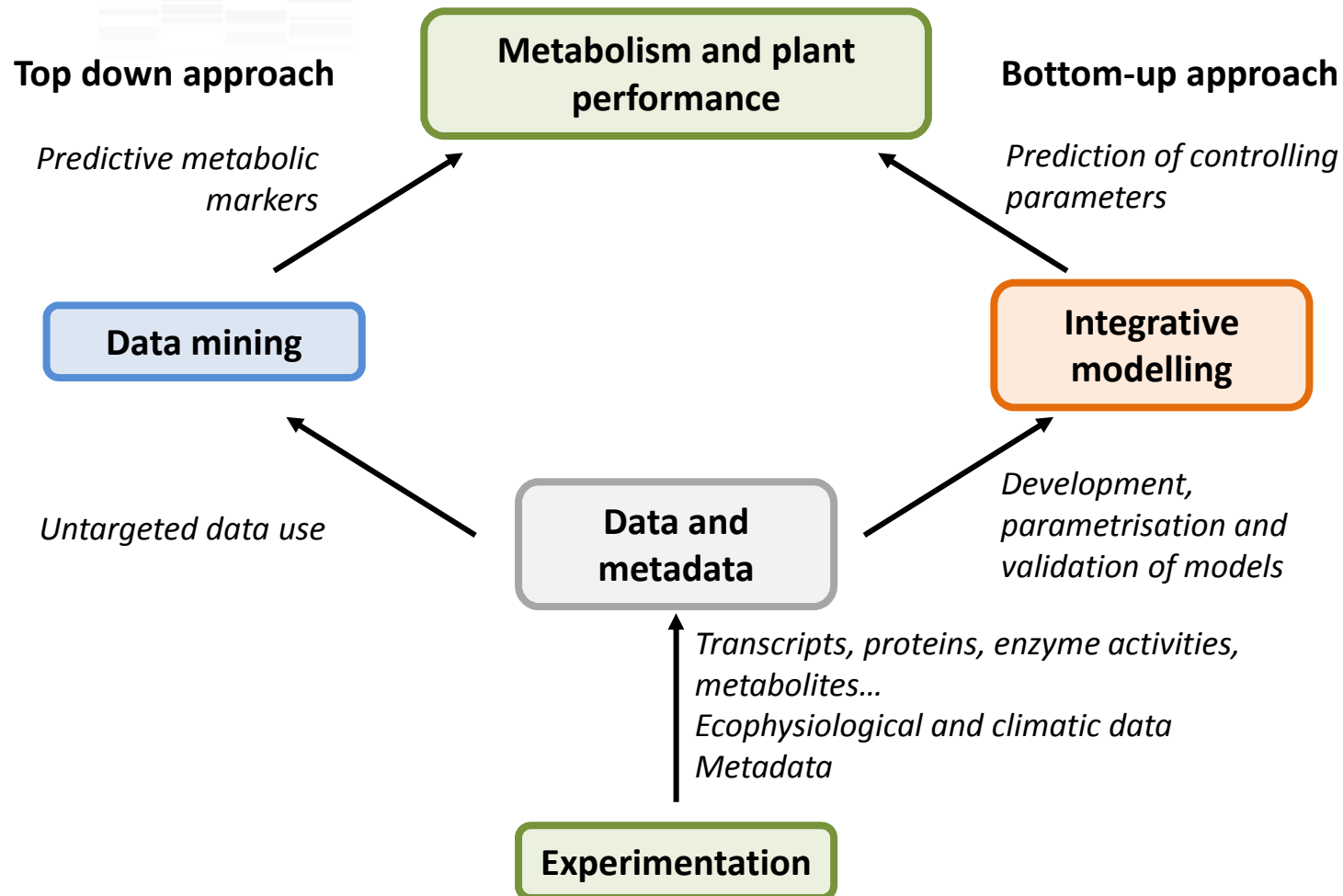
Transgenesis and yield: what are our targets?

Helen L. Jenner

Sainsbury Laboratory, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK

Attempts to increase yield of some agronomically important crops using this approach have highlighted the inherent complexities of modulating plant metabolism.

Scientific strategy





The top down approach

From metabolic traits to plant performance to genes

Metabolic traits as powerful biomarkers in maize

Genomic and metabolic prediction of complex heterotic traits in hybrid maize

Christian Riedelsheimer¹, Angelika Czedik-Eysenberg², Christoph Grieder¹, Jan Lisec², Frank Technow¹, Ronan Sulpice², Thomas Altmann³, Mark Stitt², Lothar Willmitzer^{2,4} & Albrecht E Melchinger¹

Table 1 Summary of whole-genome and metabolic prediction

GCA	h^2_{GCA}	w_M^2	SNPs			Metabolites		
			$r_{(y,y)}$	$r_{(g,g)}$	s.d.	$r_{(y,y)}$	$r_{(g,g)}$	s.d.
Dry matter yield	0.89	0.73	0.74	0.78	0.07	0.48	0.60	0.11
Plant height	0.95	0.72	0.70	0.72	0.06	0.52	0.63	0.10
Dry matter concentration	0.96	0.72	0.78	0.80	0.07	0.66	0.79	0.06
Female flowering	0.98	0.71	0.80	0.81	0.06	0.67	0.80	0.07
Starch content	0.93	0.73	0.70	0.73	0.07	0.59	0.71	0.07
Sugar content	0.94	0.74	0.69	0.72	0.06	0.55	0.67	0.09
Lignin content	0.82	0.73	0.72	0.80	0.05	0.50	0.64	0.10

Predictive abilities $r_{(y,y)}$ and prediction accuracies $r_{(g,g)}$ averaged over all cross-validation runs and their s.d. are shown for models using either SNPs or metabolites. Heritabilities of the predicted traits (h^2_{GCA}) are given as well as the repeatabilities of the used metabolic profile (w_M^2) calculated as the weighted sum of the repeatabilities of the individual metabolites (see Online Methods).

- 285 x 2 hybrids grown in several fields
- 130 metabolites measured at an early vegetative stage
- 56k SNPs
- Both groups provide prediction accuracies of up to 80% for agronomical traits using ridge regression—best linear unbiased predictions (RR-BLUP)
- Single metabolites correlate only weakly
- **Predictions were made within an experiment**

Can we predict agronomical traits using metabolic phenotyping?



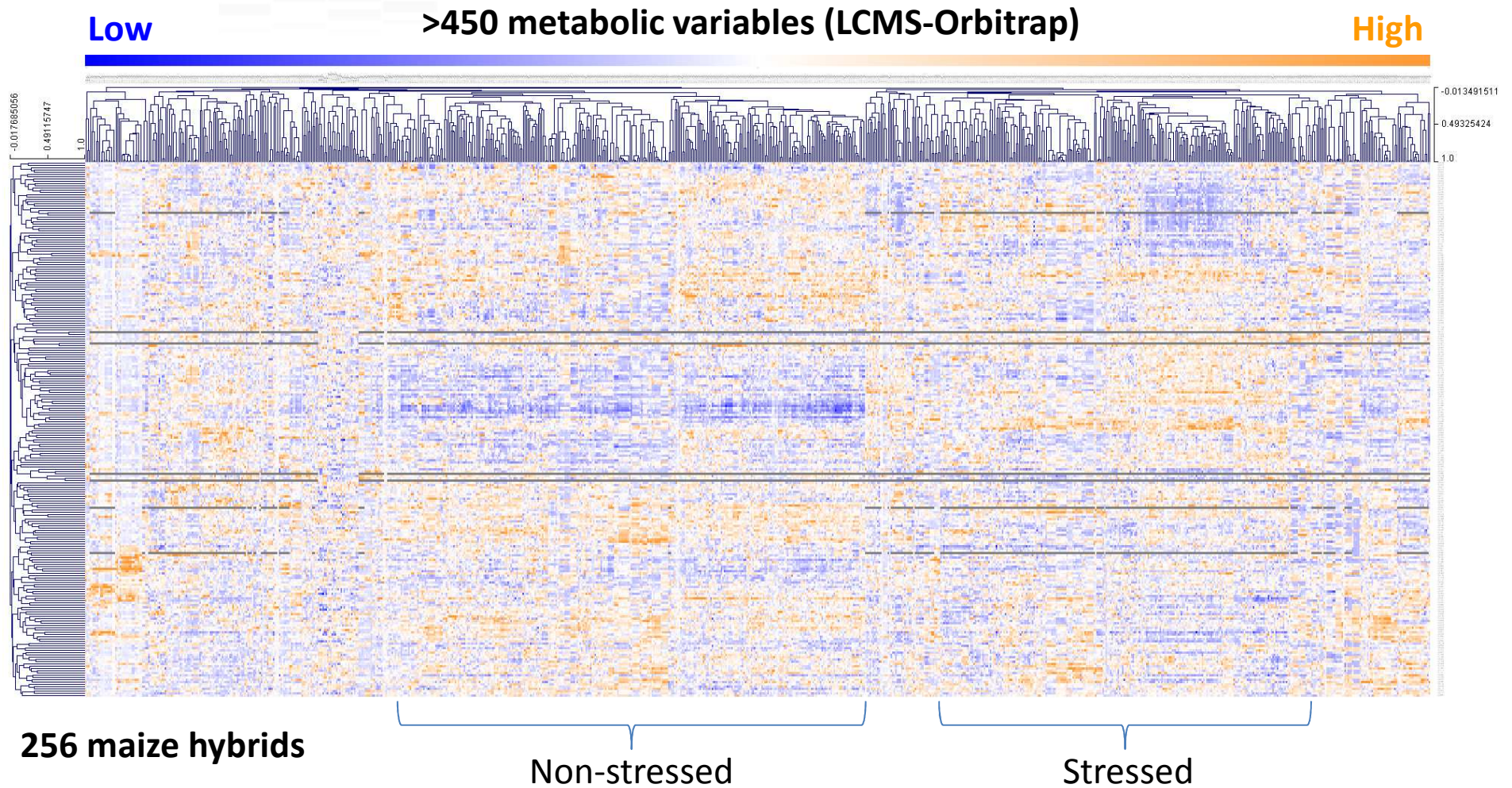
Maize hybrids grown at Phenoarch
in Montpellier
-> **Leaf samples** -> **Metabolomics**



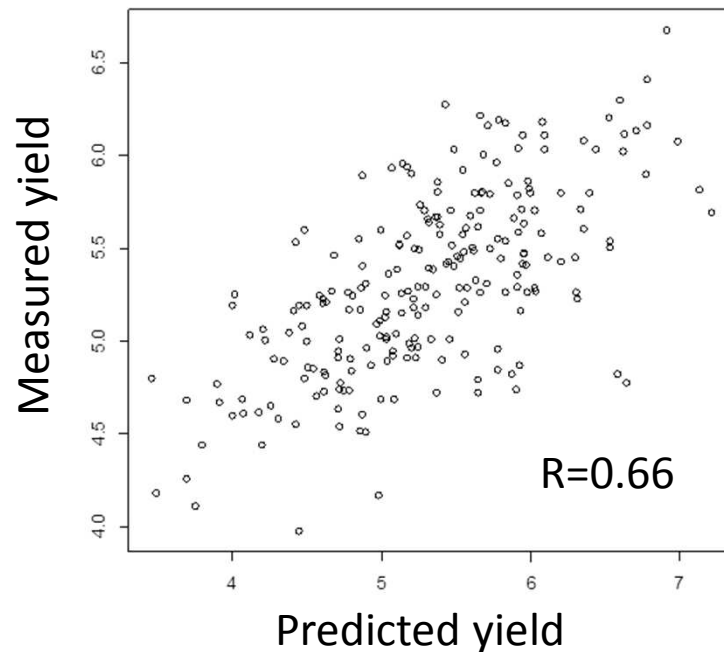
Same genotypes evaluated
in the field
-> **Yield**



Untargeted metabolic profiling in maize leaves



Metabolomics-based yield prediction



Partial least squares regression:

- **256 maize hybrids**
- **Input:** 230 metabolic variables measured in leaf samples collected in the greenhouse
- **Output:** average yield under optimal conditions (14 field experiments)

- ▶ Predict yield for various growth scenarios
- ▶ Use linear models in order to select the best biomarkers
- ▶ Search for QTLs



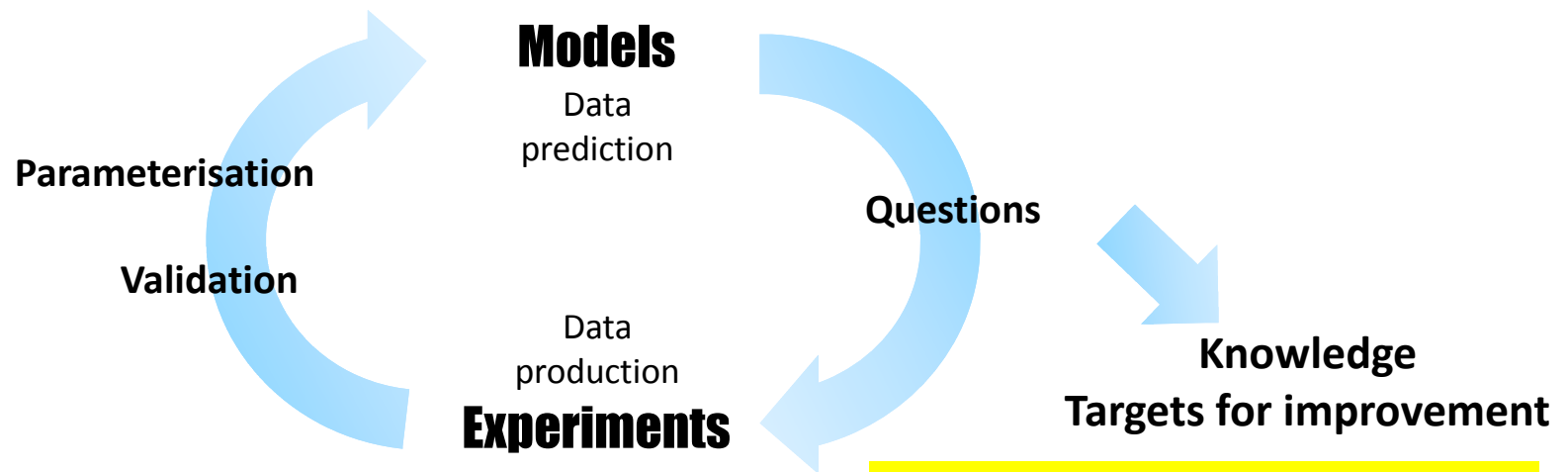
The bottom-up approach

From modelling to parameters to plant performance

Systems Biology

- Stoichiometric
- Kinetic
- Ecophysiological

- Statistical analysis



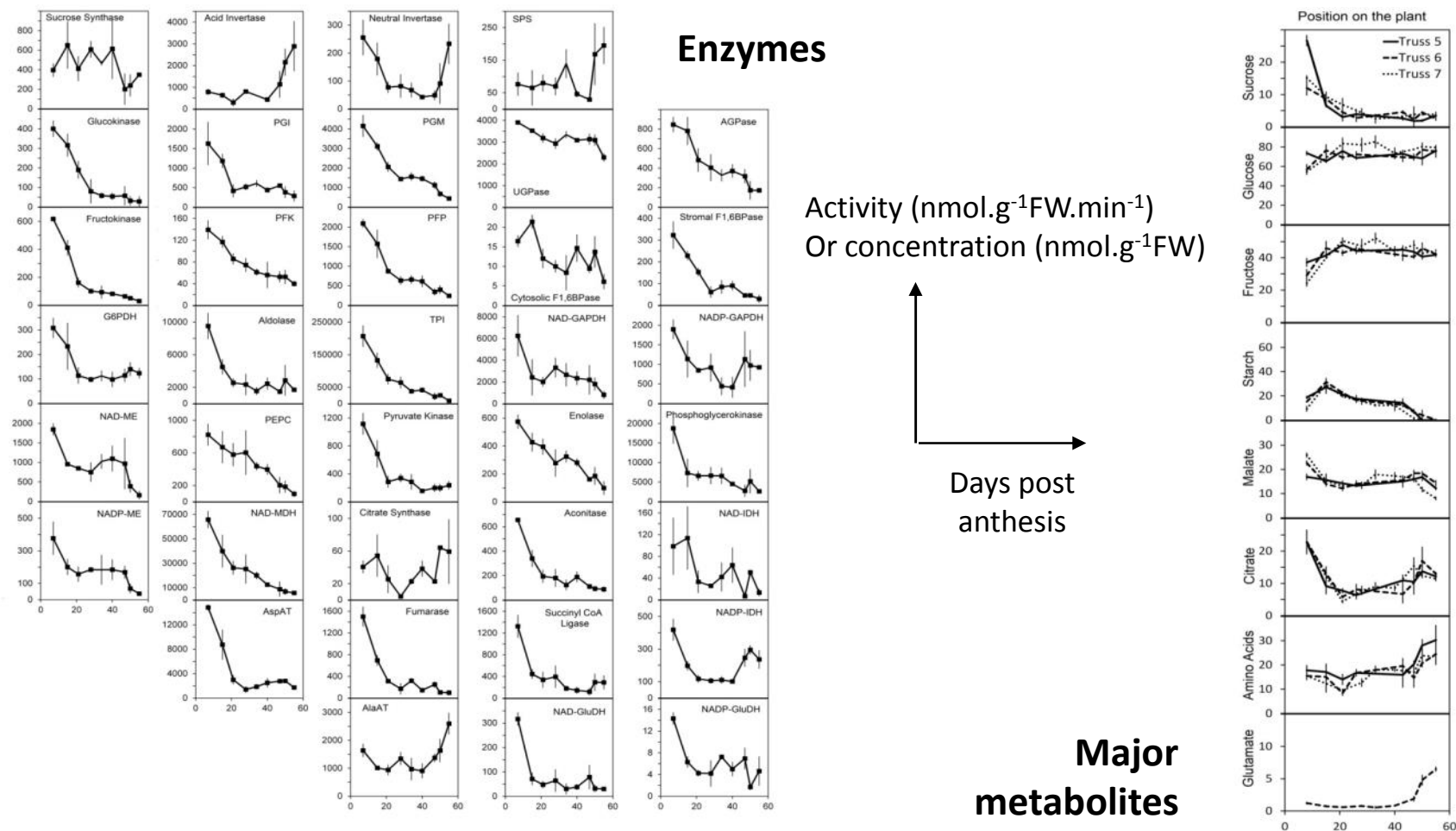
- Parameters (enzyme activities...)
- Internal variables (metabolic intermediates...)
- External variables (biomass composition)

- Fruit as the model system

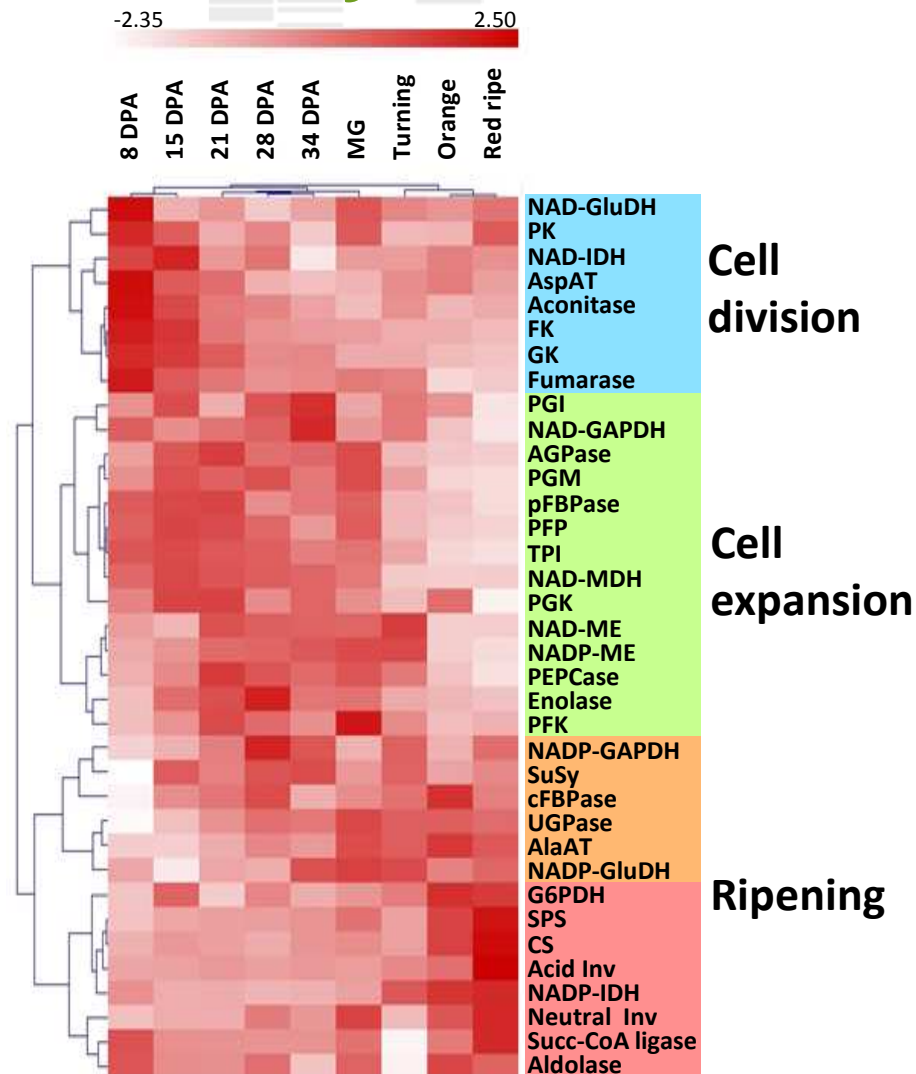
- Plant and fruit growth
- Climatic and ecophysiological data
- Collection of fruit samples



Metabolic profiles throughout fruit development

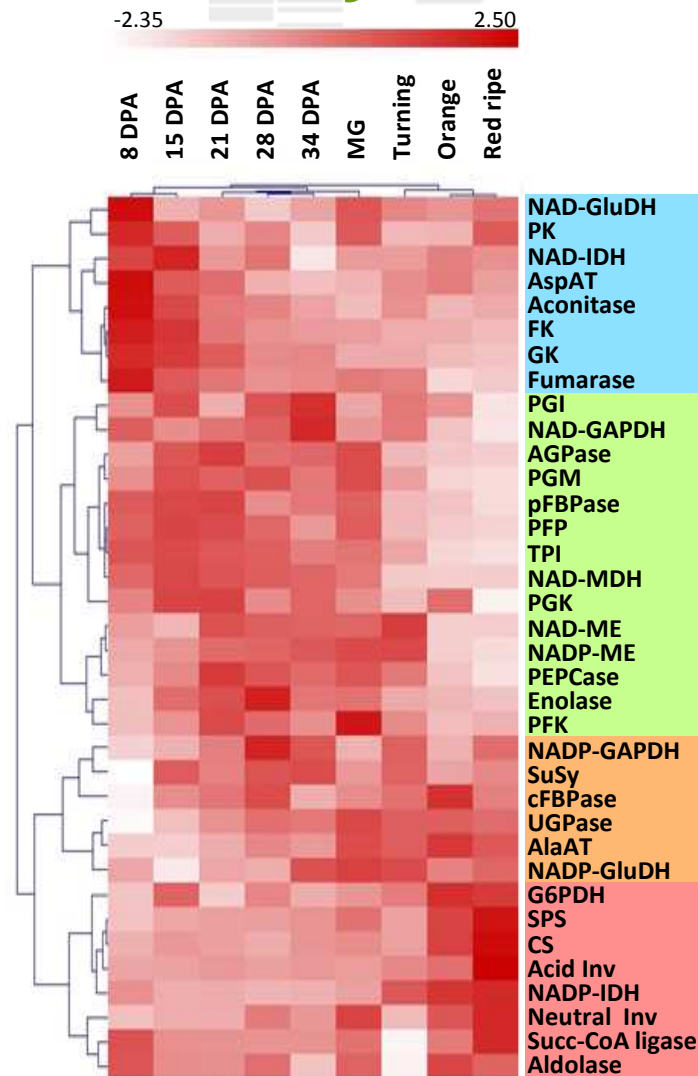


Enzyme shifts in developing fruits



Hierarchical clustering analysis
Activities expressed on protein basis
Scaled Mean centered data
Pearson correlation

Enzyme shifts in developing fruits

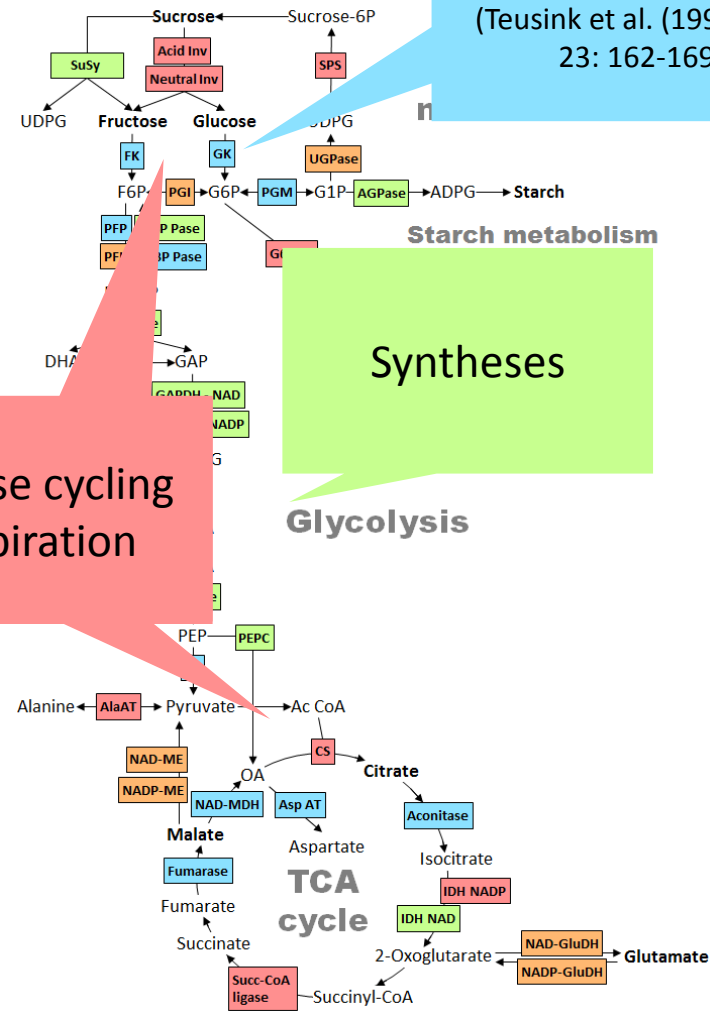


Cell division

Cell expansion

Ripening

Sucrose cycling
Respiration



Turbo metabolism
(Teusink et al. (1998) TIBS
23: 162-169)

Ecophysiological modelling to link carbon demand and growth using TOMGRO

$$\frac{dC}{dt} = \frac{dDW}{dt} (CDM + Qg) + Q_m \cdot DW \cdot Q_{10}^{(T-30)/10}$$

Diagram illustrating the components of the TOMGRO model equation:

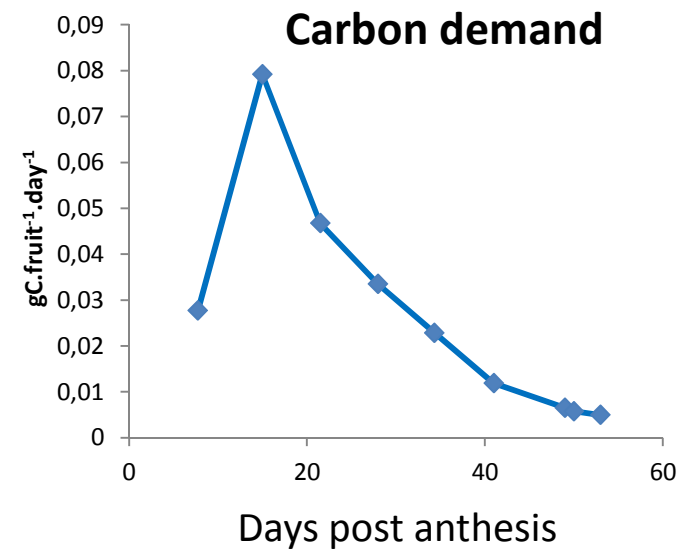
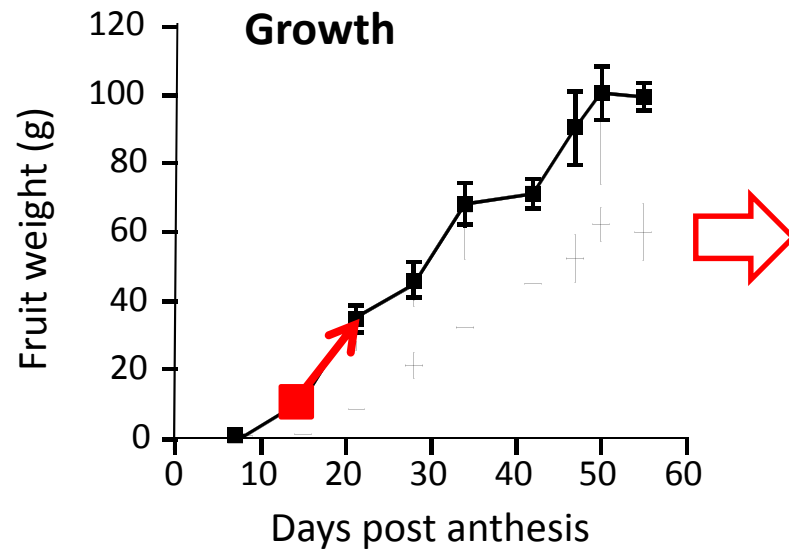
- $\frac{dC}{dt}$: Carbon demand
- $\frac{dDW}{dt}$: Biomass
- CDM : Carbon fraction of the dry matter
- Qg : Growth respiration coefficient
- Q_m : Maintenance respiration coefficient
- $Q_{10}^{(T-30)/10}$: Temperature coefficient

Parameters, *mostly empirical*

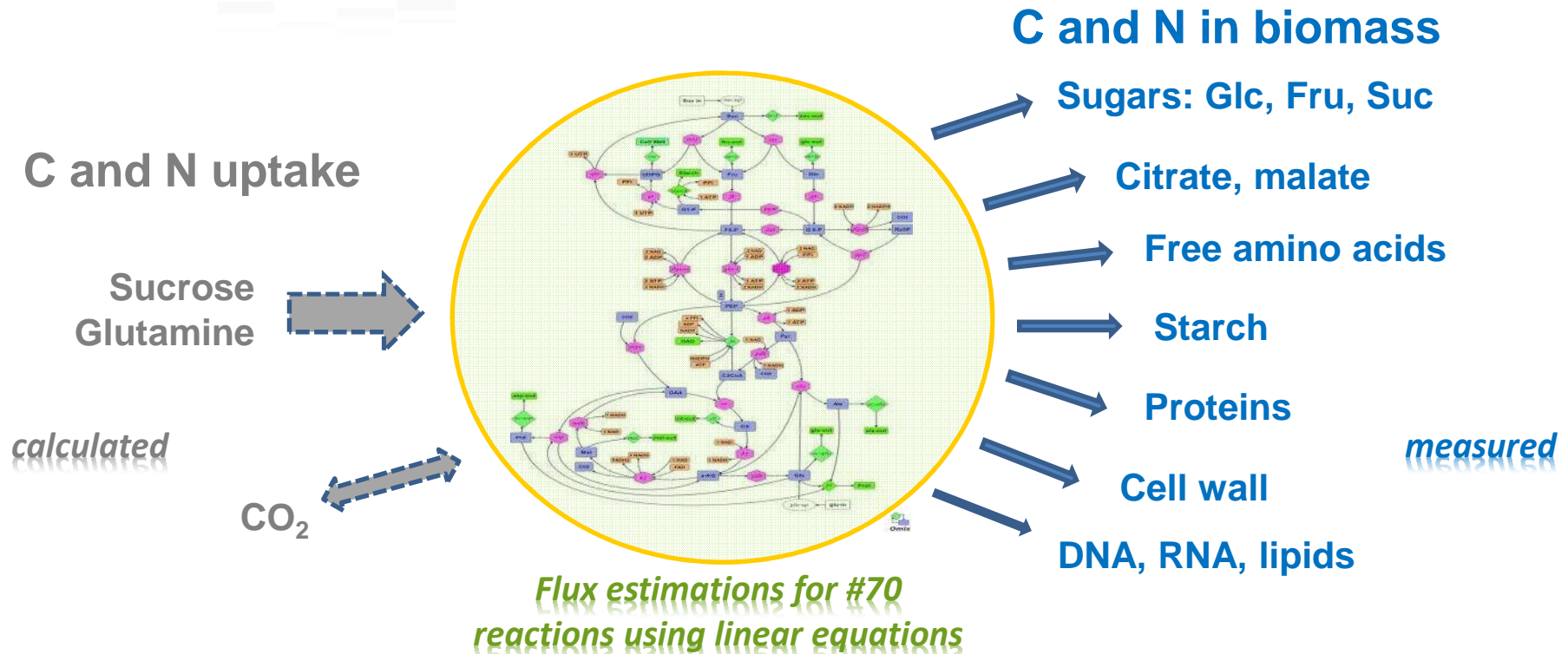
Ecophysiological modelling to link carbon demand and growth

Climatic data

$$\frac{dC}{dt} = \frac{dDW}{dt} (CDM + Qg) + Q_m \cdot DW \cdot Q_{10}^{(T-30)/10}$$



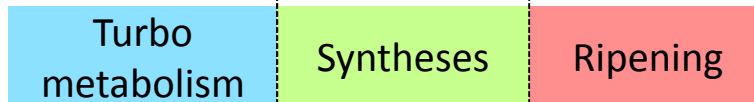
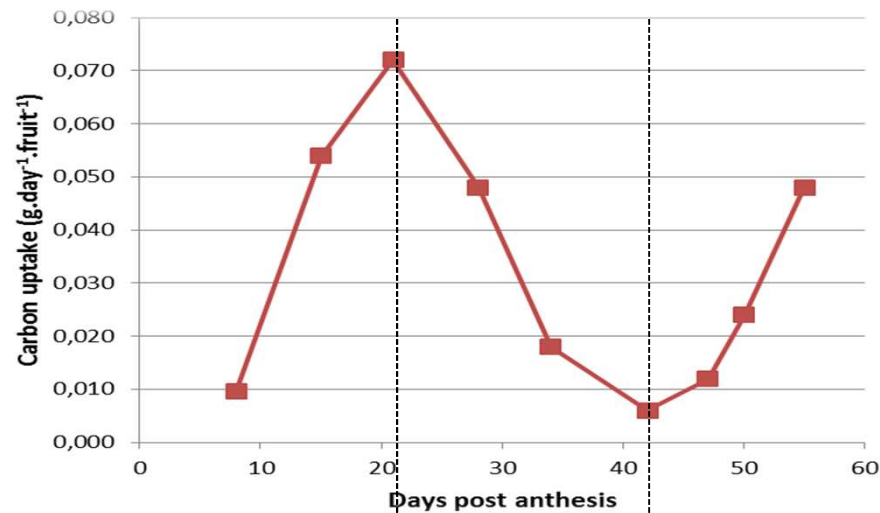
Stoichiometric model of fruit central metabolism



Assumptions:

- All cells similar, pericarp homogenous
- Steady state at each of the 9 stages of development
- Objective function= Principle of flux minimization (Holzhütter 2004)

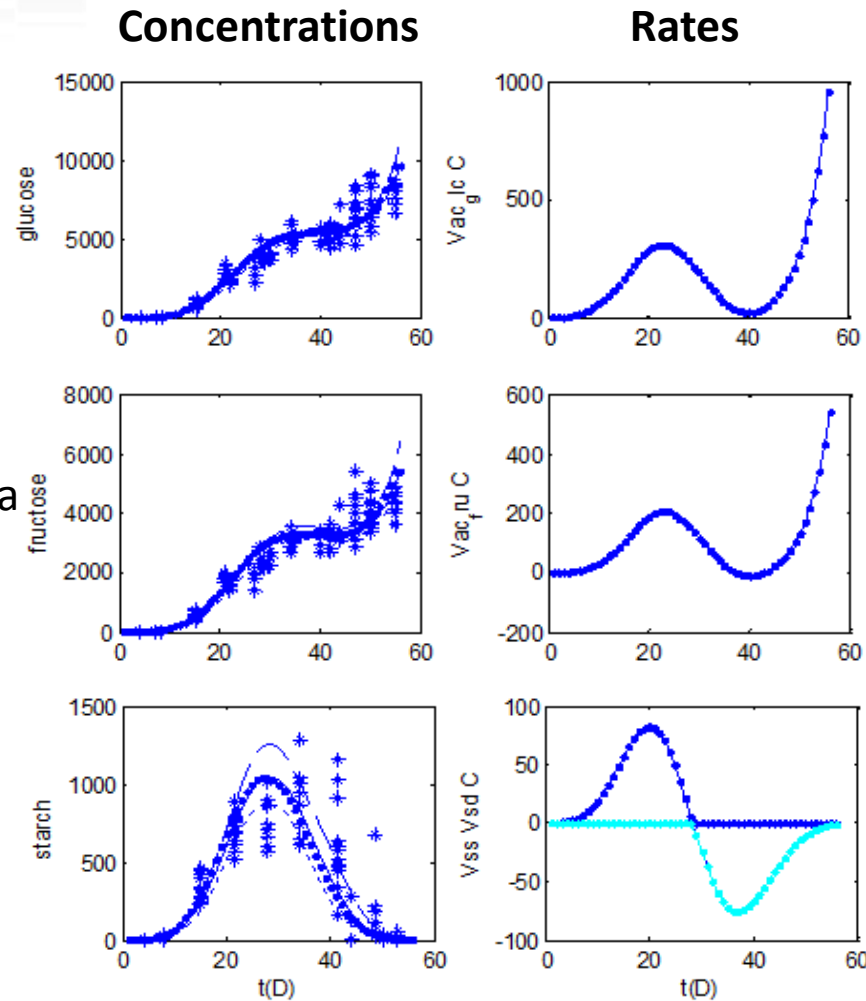
Estimation of the carbon demand



Prediction matches interpretation of enzyme profiles

Calculating fluxes at any time during fruit development

Functions are fitted over experimental data for all measured components

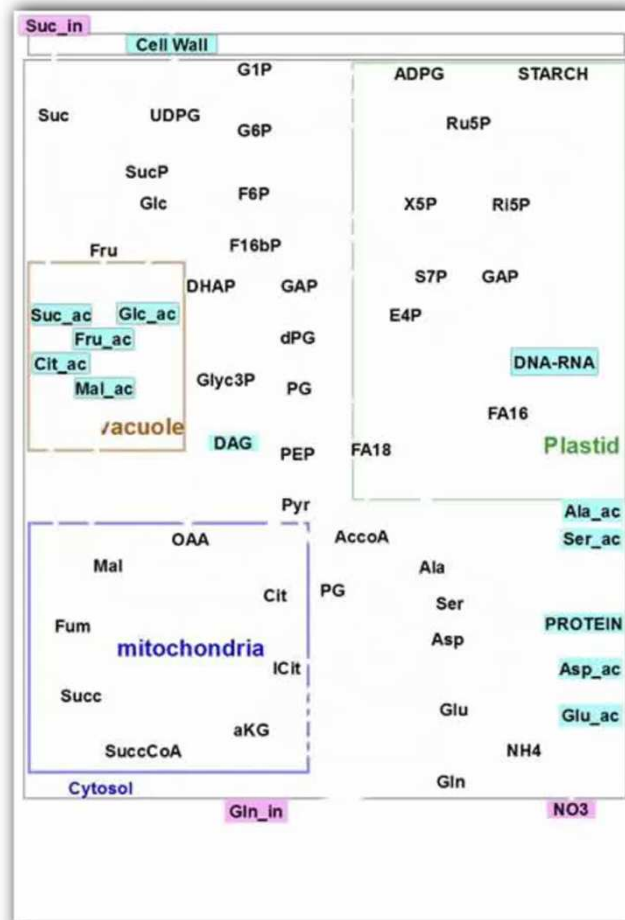


This enables the calculation of the corresponding fluxes at any time during fruit development

A time course can be considered as a succession of steady-states

Data are expressed on a fruit basis

Flux map throughout fruit development



Cell division: import of sugars and organic acids into the vacuole

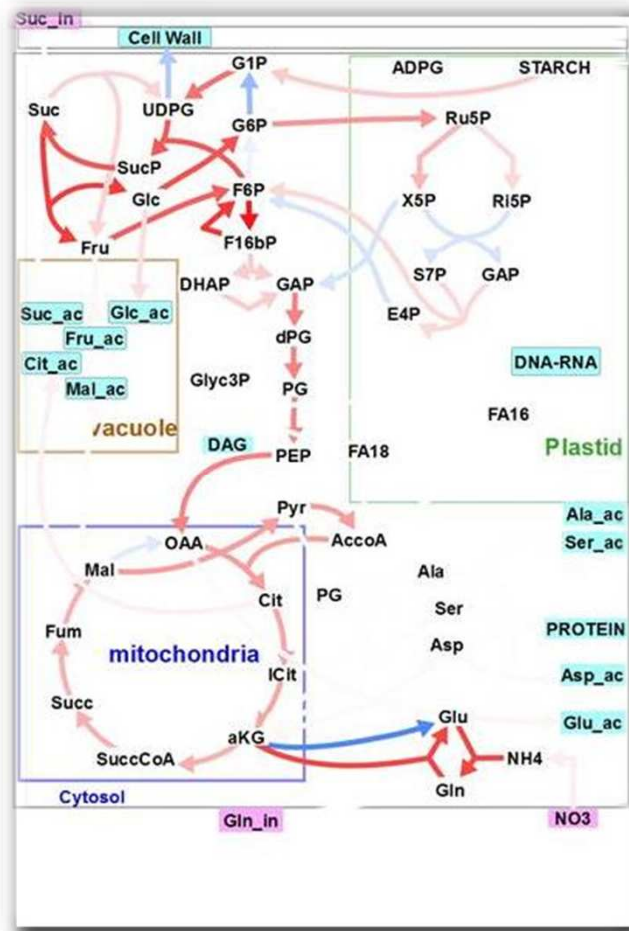
Cell expansion: slowing down of most fluxes, accumulation of starch

Breaker stage: a sudden metabolic burst following starch degradation

Ripening: reactivation of sugar and citrate import into the vacuole

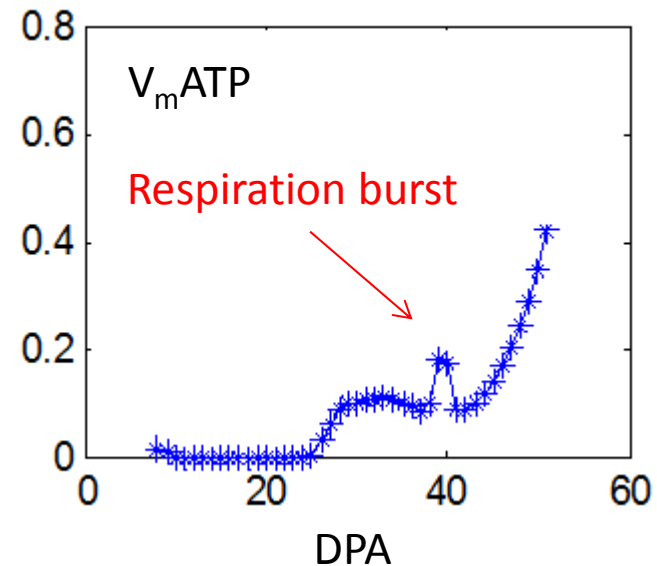
Data are expressed on a fruit basis

Respiration climacteric as an emergent property of the modelled system



ATP used for maintenance:

$$V_m \text{ATP} = V_{\text{total}} \text{ATP} - V_{\text{biomass}} \text{ATP} - V_{\text{metab}} \text{ATP}$$

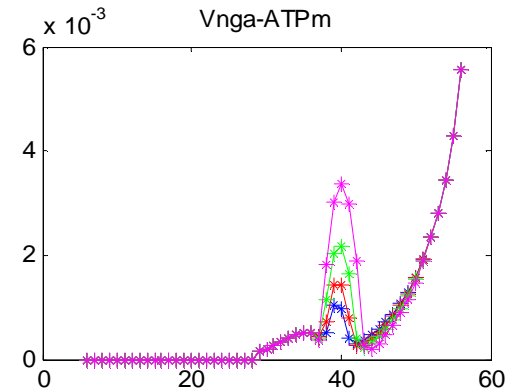
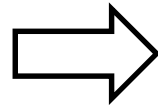
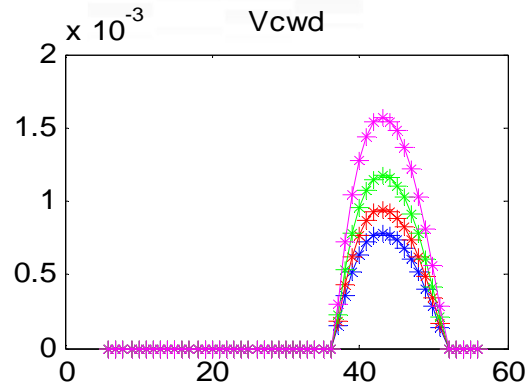


ATP is known to increase at early ripening stages in various fruits (Brady 1987)

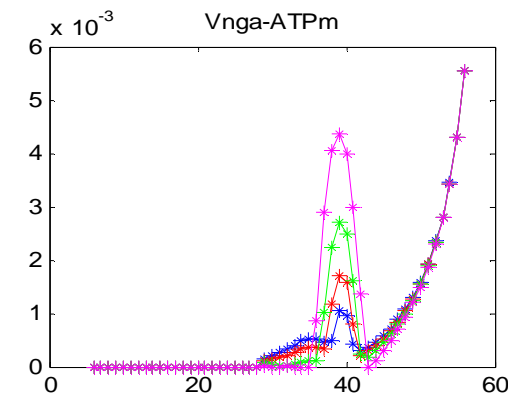
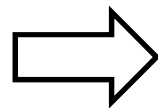
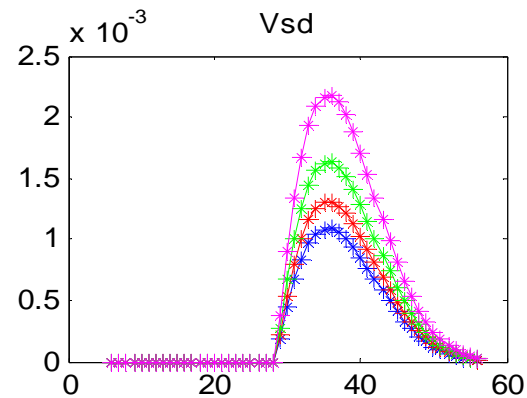
Data are expressed on a fruit basis

Starch and cell wall degradation feed the energy burst

Starch



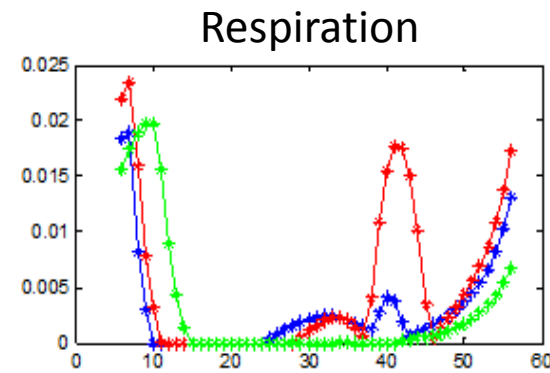
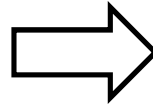
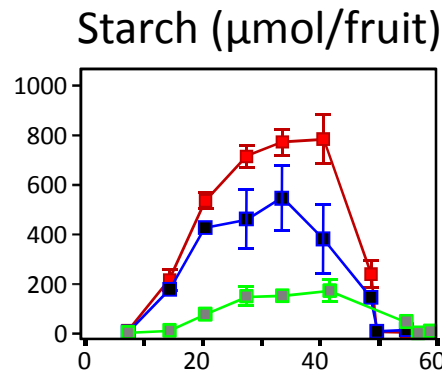
Cell wall



X2
X1.5
X1.2
Control (experimental data))

Data are expressed on a fruit basis

The respiration climacteric buffers energy



Starch is degraded very quickly, leading to a massive energy boost

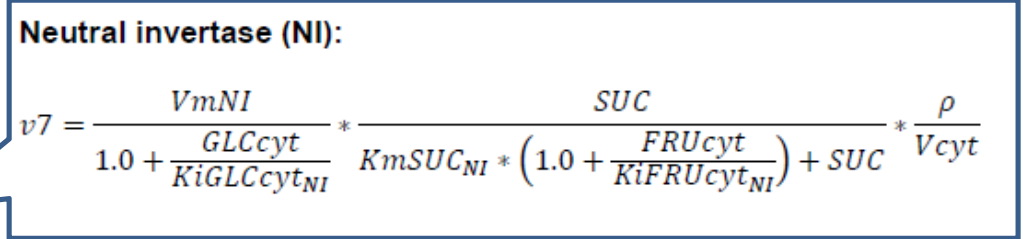
- Total protein increases and central metabolism is reprogrammed
- But there is no more growth
- Energy in excess probably needs to be dissipated...

Water stress (50% decrease in irrigation)

Control (optimal conditions)

Shading (60% decrease in photosynthetically active radiation)

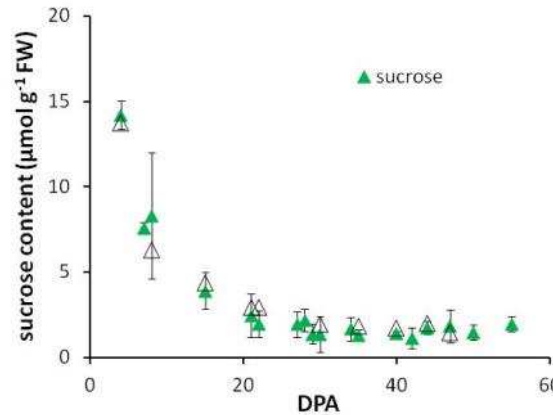
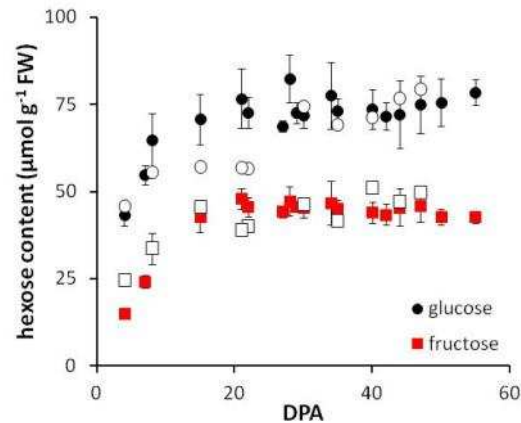
Kinetic



24 reactions: 13 ODE, 1 influx, 6 outfluxes (growth, glycolysis), 4 carriers

Sucrose metabolism in the pericarp of tomato fruits

Model optimisation

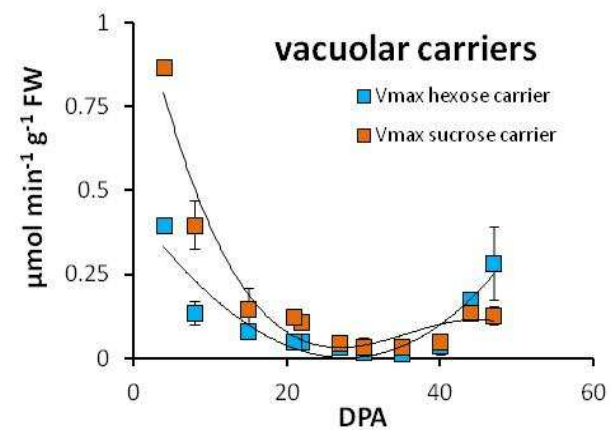
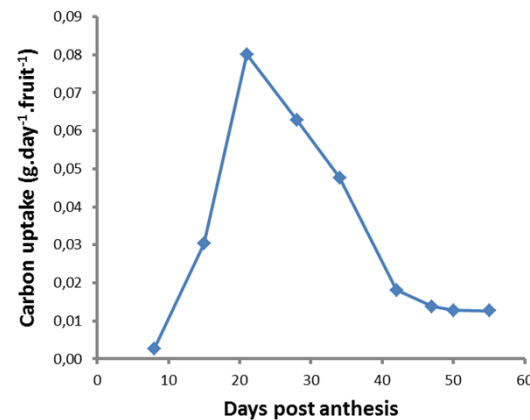


Variables

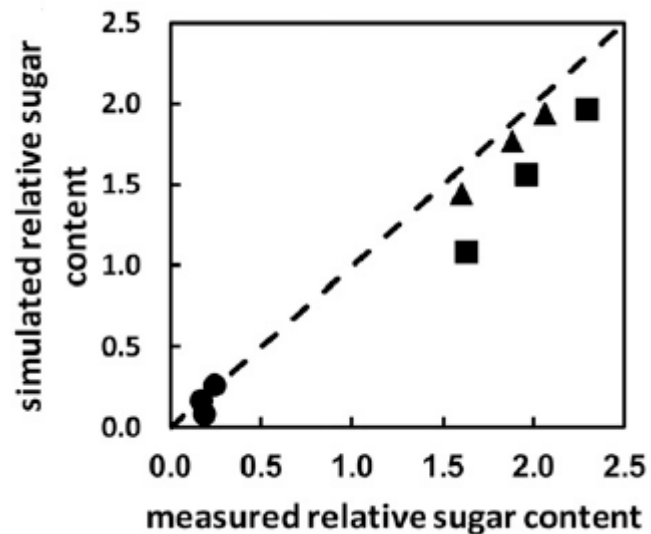
Open symbols represent predicted data

At each developmental stage, the 3 unknown parameters (C_{uptake} and sugar transport capacities) were optimized using a random search algorithm to fit the measured sugar content of the pericarp

Fitted parameters

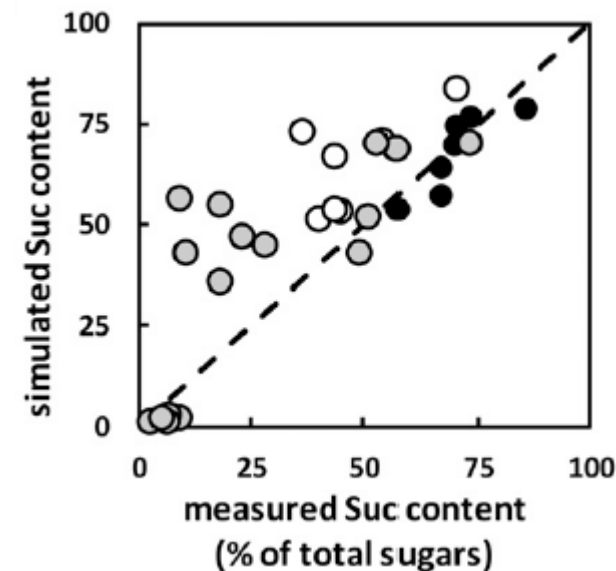


Model prediction: vacuolar invertase



- Sucrose
- Glucose
- ▲ Fructose

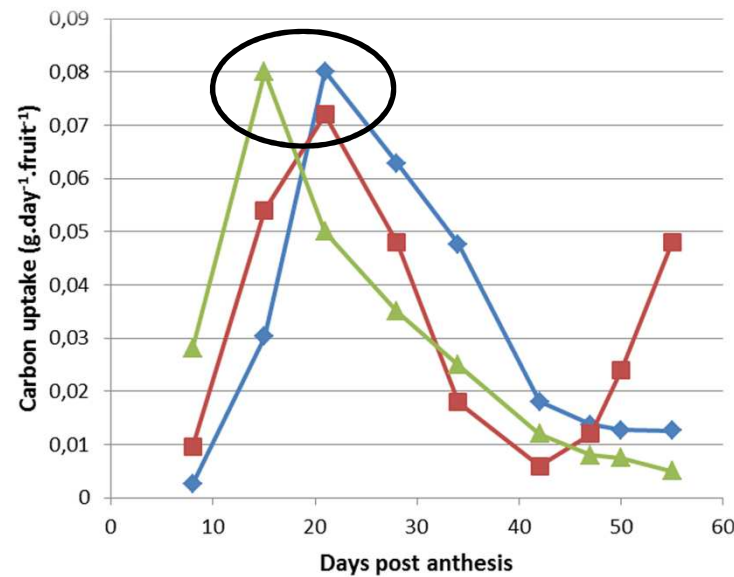
Data from Steinhauser *et al.* (2010) and Carrari *et al.* (2006); 4 stages of fruit development



- Acid invertase antisense *S. Lycopersicum*
- *Solanum chmielewskii*
- *S. Chimielewskii* x *S. Lycopersicum* introgression lines

Data from Klann *et al.* (1996) and Yelle *et al.* (1988, 1991)

Model cross-validation



0.08 g = 20 g sugar/kg/day !
An infant needs #25



Ecophysiological

Stoichiometric

Kinetic

Fruit abortion

“young tomatoes that fall before they ripen”

Message d'origine	
Stephanie C (91) 01/08/2011 à 22:40  	Good evening, I spoke with a colleague who has a serious problem with his tomato plants, they make beautiful clusters of flowers, small tomatoes grow then and they fall one after the other. As a result, he has very few tomatoes this year. Does it come from watering, weather? Have you ever encountered this problem? Thanks for your help.



A net intercepting 60% of the light (PAR) was installed during fruit growth:

- Nearly 100% of fruits at cell division stage aborted
- About 100% of fruits at cell expansion stage survived

► Improve yield by removing sinks of low value

Blossom end rot

- Non-pathogenic necrosis appearing at the bottom of growing fruits that provokes up to 50% losses
 - Calcium often incriminated but probably multifactorial: drought, high temperatures, nutrient deficiencies...
 - Turbo metabolism could be involved
- Control temperature as well as water and nutrient supply
- Breed for varieties having growing fruits with high levels of antioxidants



Acknowledgements

Metabolism Group
Fruit Biology & Pathology Unit



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Whole group

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Collaborators

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Tomato: Invenio, Jean-Pierre Mazat & Christine Nazaret (Bordeaux University)

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