Modelling Tomato Fruit Metabolism

A component of the FRIM collaboration

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http://frim.brookes.ac.uk

FRIM (**FR**uit **I**ntegrative **M**odelling) Partners

- INRA Bordeaux: PI and coordinator, Yves Gibon
- INRA Avignon: PI Michel Génard
- Oxford University Plant Sciences, PI Lee Sweetlove
- Oxford Brookes University, PI David Fell
- Université de Bordeaux: PI Jean-Pierre Mazat
- Associates: MPI Mol Plant Physiol, Golm & University of Stellenbosch

Concept

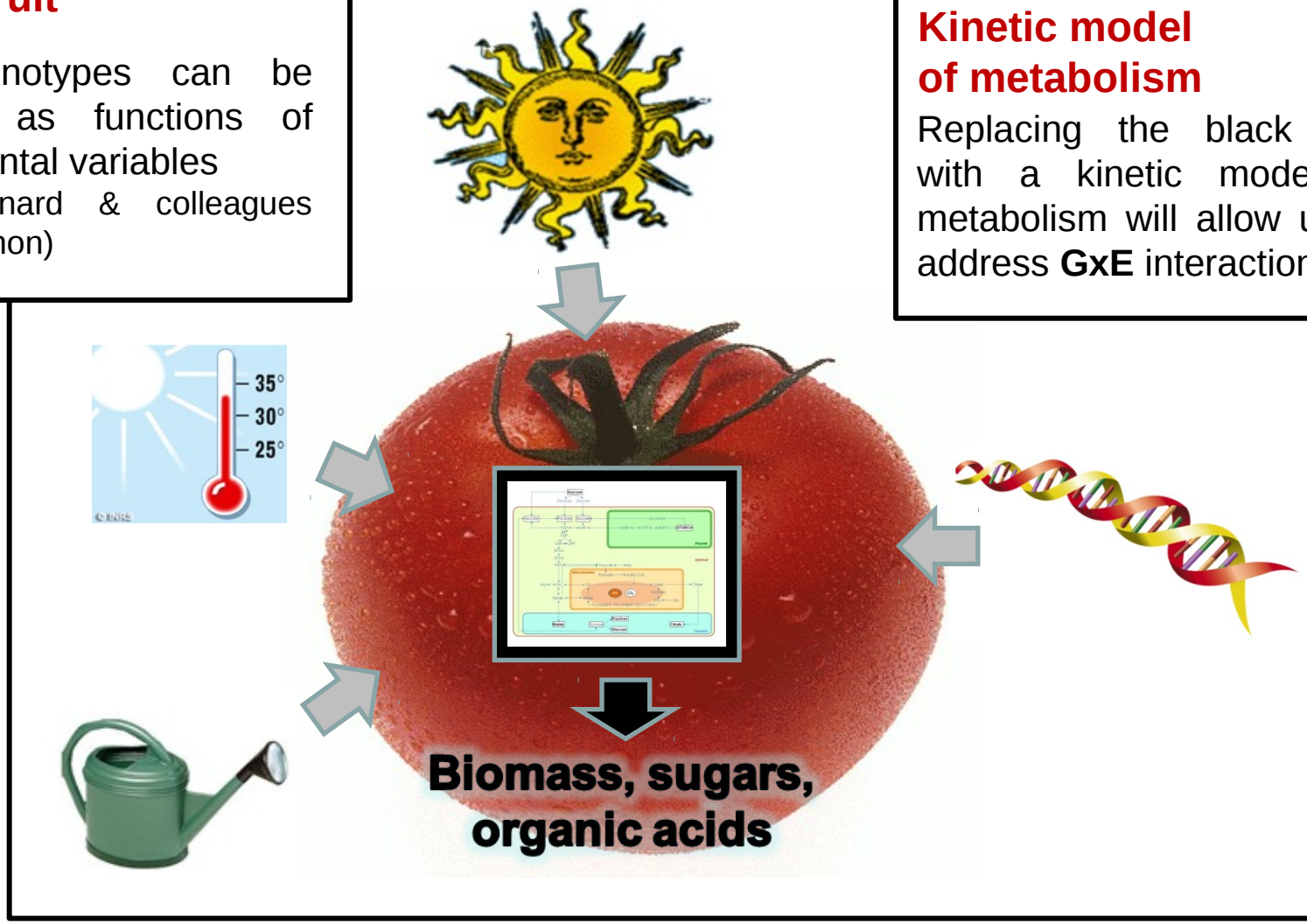
Virtual Fruit

Fruit phenotypes can be predicted as functions of environmental variables

Michel Génard & colleagues (INRA-Avignon)

Kinetic model of metabolism

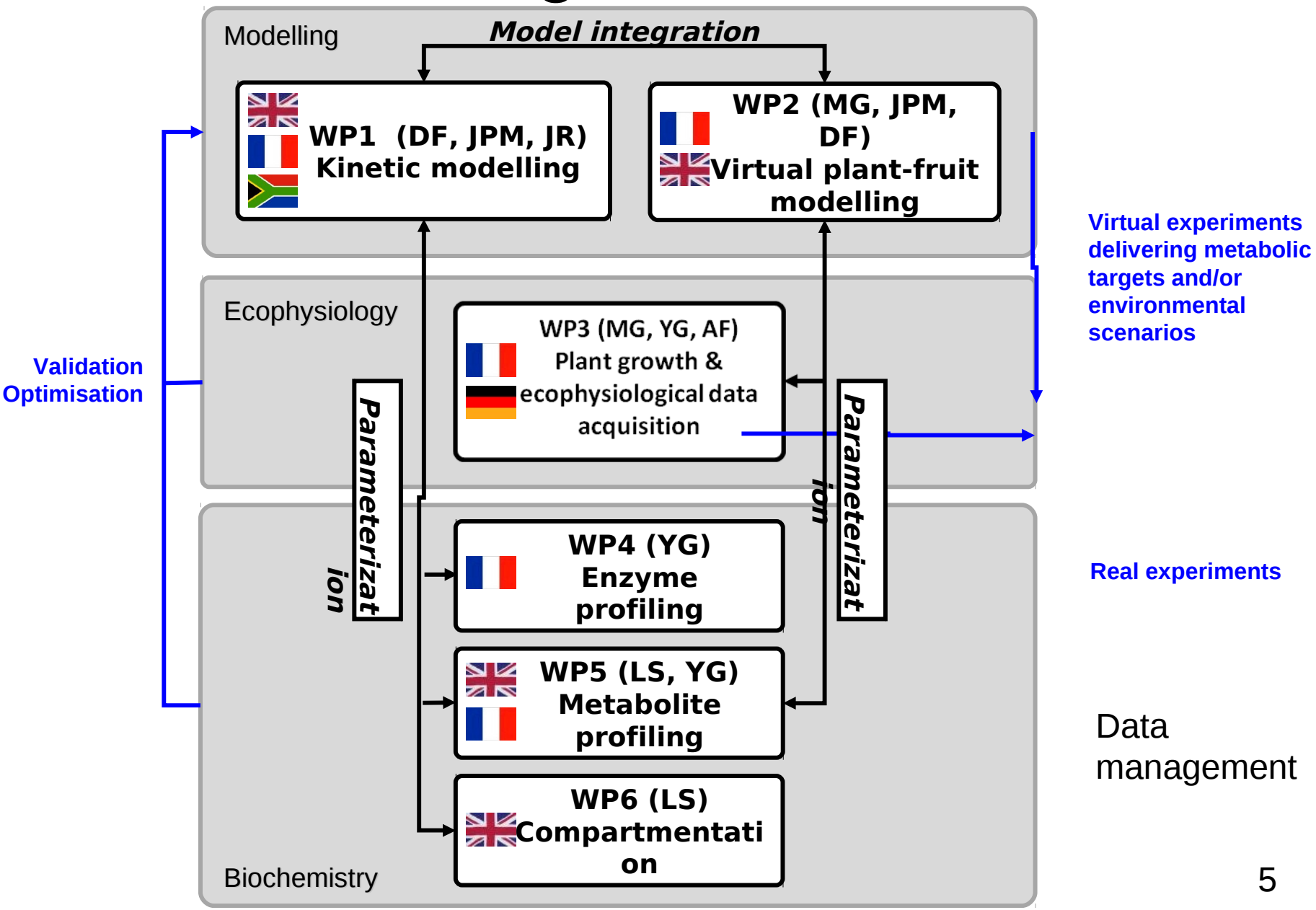
Replacing the black box with a kinetic model of metabolism will allow us to address **GxE** interactions!



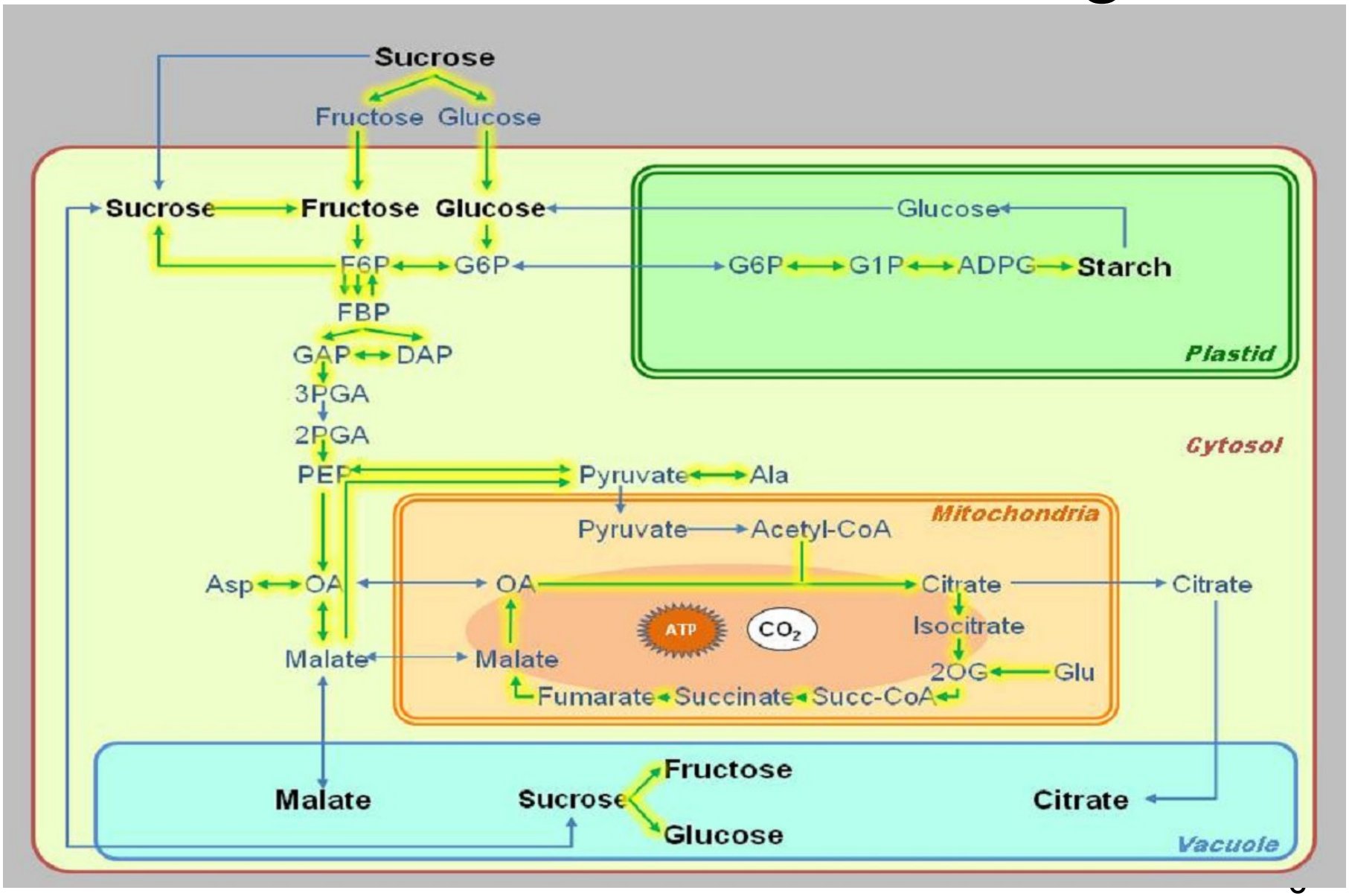
Aims

- To build a kinetic model encompassing the major routes carbon takes, once imported into the fruit cells from the source organs of the mother plant.
- To integrate the kinetic model with a phenomenological model predicting sugar and organic acid contents as functions of time, light intensity, temperature and water availability.
- To obtain large-scale experimental measures of the consequences of altered environmental conditions.

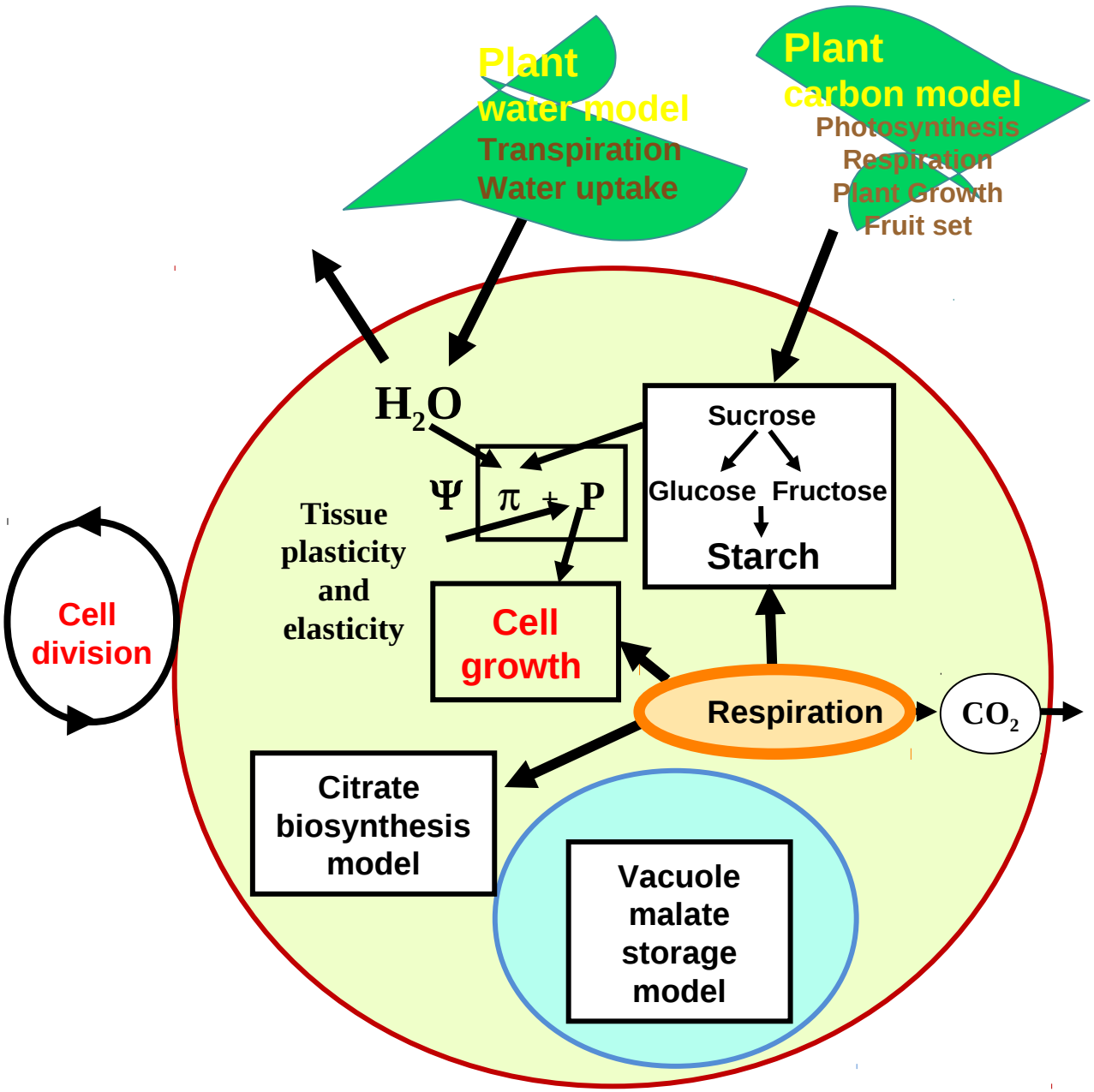
Organisation



WP1: Kinetic modelling



WP2: Virtual Plant-Fruit Modelling



WP3: Eco-physiological data

Year 1

- Contrasted growth scenarios:
  - -optimal
  - -low light intensity
  - -water stress
  - -high temperature
  - -Low temperature
- Monitoring of the major environmental variables
- Harvests at different developmental stages (80 plants per experiment required)

Years 2-3 Validating experiments with transgenic plants and selected growth scenarios

WP4: Enzyme Profiling

- 50 enzyme activities
  - Across fruit development
- Kinetic properties (V_{total}, K_m, K_i) of enzymes predicted to have strong control coefficients
- See the robot in action at: http://frim.brookes.ac.uk/video

	Substrate	Km µM	
		Arabidopsis	BRENDA
Aconitase	Aconitate	17 ± n.d.	30-100
AGPase	ADPG	153 ± n.d.	190*
Fumarase	Fumarate	145 ± n.d.	-
G6PDH	G6P	162 ± n.d.	150
GAP DH (NAD)	3PGA	391 ± 49	-
	NADH	235 ± 29	-
GAP DH (NADP)	3PGA	174 ± 100	-
	NADPH	73 ± 7	-
Glycerate Kinase	Glycerate	250 ± 28	250
	ATP	166 ± 10	220
Glycerol kinase	ATP	115 ± n.d.	-
Glutamine synthetase	Glu	1546 ± n.d.	3000
Isocitrate DH (NADP)	Isocitrate	12 ± 1	6-40
	NADP	7 ± 1	3-10
Glutamate DH (NAD)	2-OG	1684 ± 33	1700
	NADH	48 ± 1	-
PEP Carboxylase	PEP	31 ± n.d.	60-120
Phosphofructokinase (PPi)	PPi	23 ± n.d.	14-33
	F6P	98 ± n.d.	50-300
Phosphoglucose Isomerase (plastidial)	F6P	501 ± n.d.	480
Phosphoglucose Isomerase (cytosolic)	F6P	247 ± n.d.	230
Phosphoglycerokinase	3PGA	834 ± n.d.	600-1100
	ATP	326 ± n.d.	150-4000
Pyruvate kinase	PEP	73 ± 23	50-120
	ADP	219 ± 34	75-240
RubisCO (initial)	RuBP	25 ± n.d.	10-120
RubisCO (activated)	RuBP	23 ± n.d.	10-120
Shikimate DH	Shikimate	235 ± n.d.	28-200
	NADP	5 ± n.d.	7-25
Sucrose Phosphate Synthase	UDPG	3994 ± 203	3000
	F6P	1880 ± 173	1400

The Bordeaux robot

WP5: Metabolite Profiling

- GC-MS and NMR for a broad range of primary metabolites including sugars and organic acids.
- LC-MS, spectrophotometric, and luminometric assays for targeted “key” metabolites
  - phosphorylated intermediates
  - acetyl-CoA and CoASH
  - ATP/ADP/AMP
  - NAD/NADH

WP6: Compartmentation

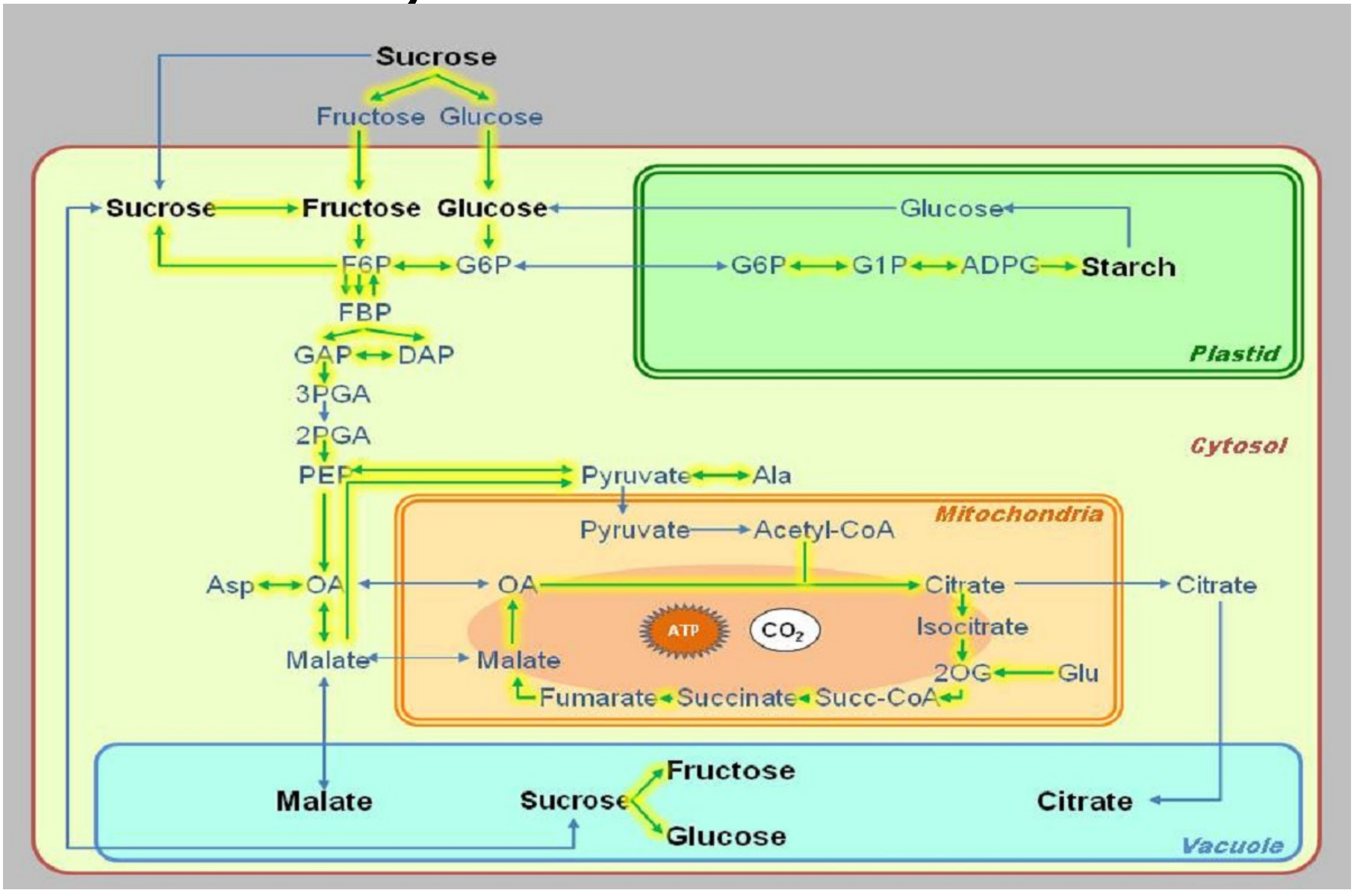
- kinetics of tonoplast transport of sugars and organic acids
- kinetics of mitochondrial transport of sugars and organic acids
- quantify subcellular compartment volumes

The kinetic model

The model (currently cytosolic reactions only) is simulated with the ScrumPy package using “minimal” enzyme kinetic functions:

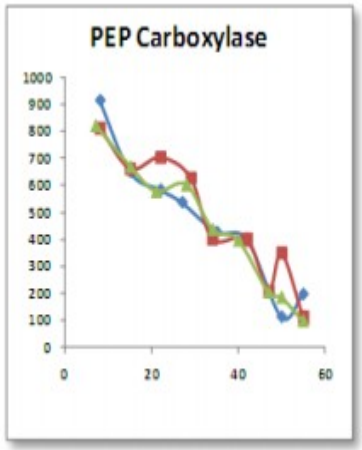
```
Inv:
    Scr + X_H2O <> Glc + Fru
    (Vmax_Inv)*(Scr-Glc*Fru/Keq_Inv)/
     (
        (Km_Scr_Inv+Scr)+(Ki_Fru_Inv+Fru)*(Ki_Glc_Inv+Glc)
        )
    Vmax_Inv=125
    Keq_Inv=100
:
FruK:
    Fru + ATP <> F6P + ADP
    Vmax_FK*(Fru*ATP-F6P*ADP/Keq_FK)/
     ((Km_FK_Fru*(1+F6P/Km_FK_F6P)+Fru)*(Km_FK_ATP*(1+ADP/Km_FK_ADP)+ATP))
    Vmax_FK=88
    Keq_FK=1000
:
GluK:# Hexokinase                    G6P   = Glucose 6-Phosphate
    Glc + ATP <> G6P + ADP
    Vmax_HK*(Glc*ATP-G6P*ADP/Keq_HK)/
      ((Km_HK_Glc*(1+G6P/Km_HK_G6P)+Glc)*(Km_HK_ATP*(1+ADP/Km_HK_ADP)+ATP)).
```

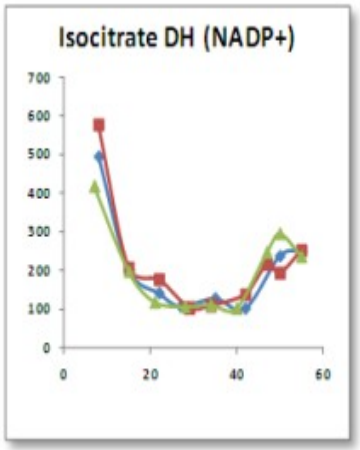
Current model corresponds to the cytosolic reactions

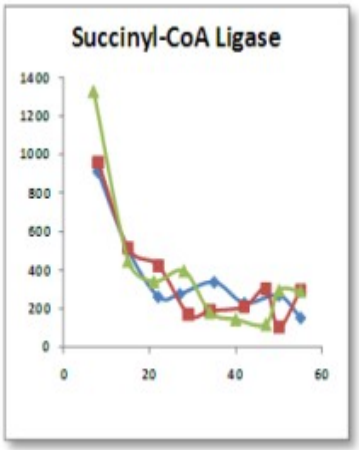


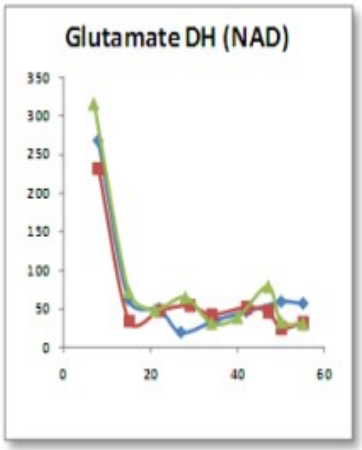
Many V_{max} values have been measured in Bordeaux

- 35 enzymes have had V_{max} values measured throughout fruit development.
- The measurements are highly reproducible.
- Virtually all enzyme follow one of the time courses shown below.





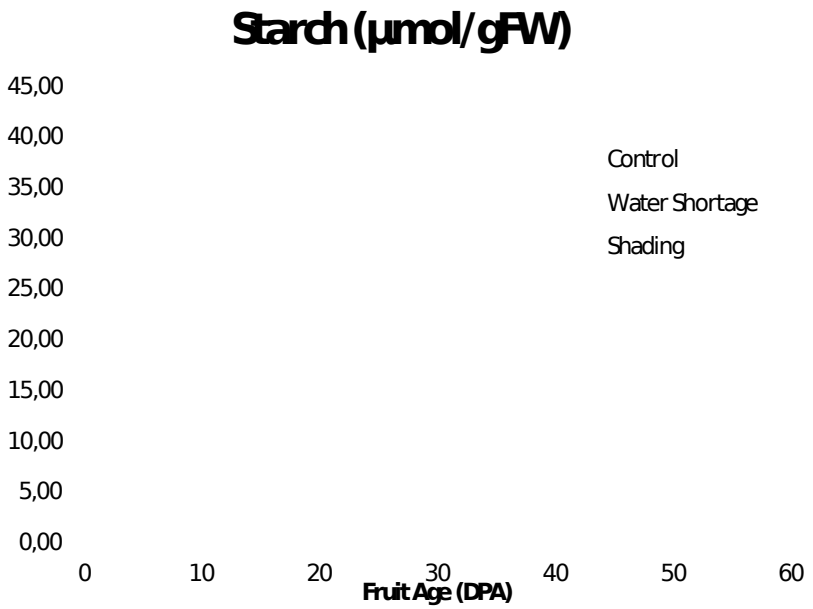


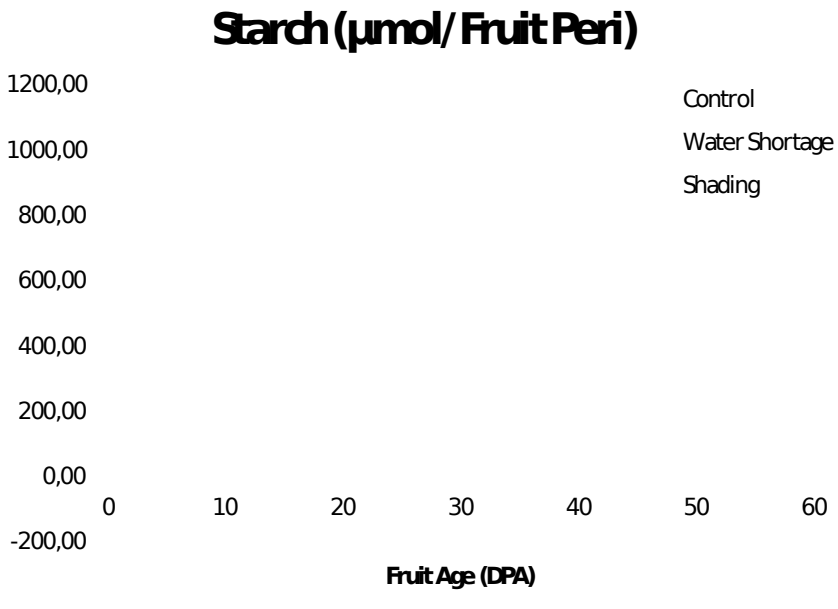


Truss 5
Truss 6
Truss 7

Clusters of enzyme responses

Concentrations or amounts?



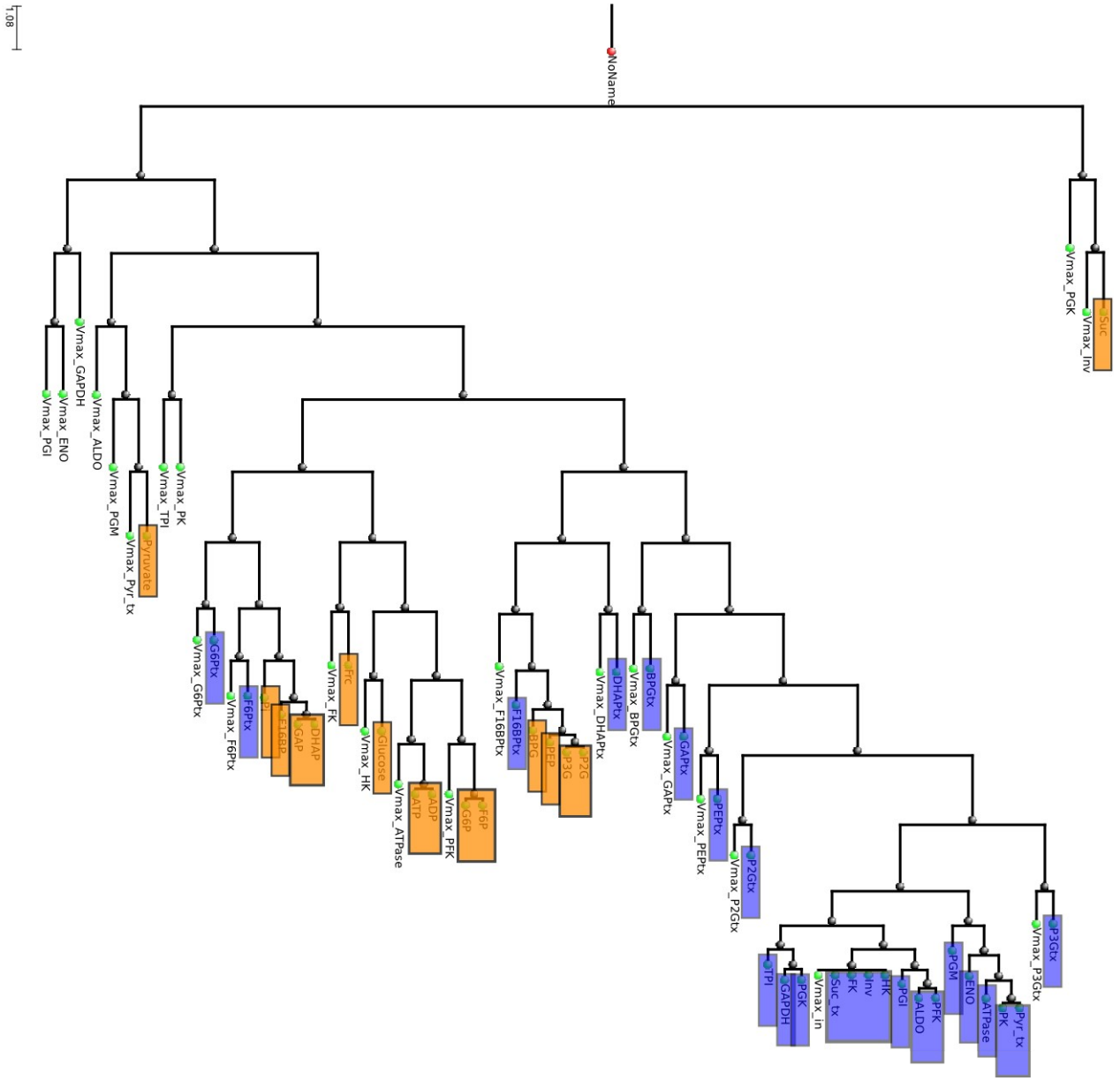


Simulating across the range of Vmax values

- All the enzymes are showing nearly 10x variation through fruit development; the changes are much larger than assay and sample variability.
- We have modelled the consumption of sucrose by cytosolic metabolism at average values of the Vmax values at different stages of fruit development.
- The metabolic model is capable of reaching steady state throughout, given suitable vales of cytosolic ATP hydrolysis, removal of sucrose to the vacuole and withdrawal of glycolytic intermediates for biosynthesis.

Responses to enzyme variation

- The experimental measurements show correlations in enzyme activities.
- To explore the role of each enzyme, we simulated with random, independent combinations of V_{max} values drawn from the measured range for each enzyme.
- From a large data set of solutions, we examined correlations between V_{max} values and steady-state variables: fluxes and metabolite concentrations.



Tree from model results

Blue: variable fluxes

Orange: variable metabolites

Interpretation

- Fluxes in glycolysis are most strongly correlated to the invertase V_{max}.
- This is consistent with a strong response of the fluxes to external sucrose concentration.
- There is strikingly little correlation between metabolite concentrations and metabolic fluxes.
- Some glycolytic enzymes (e.g. PFK) correlate with some of the glycolytic metabolite concentrations.
- A number of glycolytic enzymes show no strong correlation with either metabolite concentrations or fluxes.

Next steps in modelling

- Add additional metabolic reactions to the model, e.g. starch metabolism (available in outline from our existing potato model).
- Add the vacuolar transport reactions for storage.
- Model the transition from starch deposition up to ~ day 40 and the rapid breakdown afterwards.
- Apply Metabolic Flux Analysis to the detailed time series measurements of fruit composition to quantify the major net fluxes.
- Incorporate the metabolic model as a component of the fruit model, solving the steady state of the latter for each time step in the former (~1 hr).

Conclusion

- A 'data-rich' project: data is being collected systematically on an industrial scale to meet the needs of model development.
- Multiscale modelling: embedding the detailed metabolic model in the higher level fruit model should enable us to predict the effect of modifying enzyme activities on the properties of the fruit at harvest.
- Extending the scale: the fruit model will ultimately be incorporated within a tomato plant model.

Modelling and Web Site/ Data Management Acknowledgements

Mark Poolman & Patrick de Vries

Also Achuthanunni Chokkathukalam

and Aliah Hawari (Oxford University Plant Sciences)

ScrumPy is available from:
http://mudshark.brookes.ac.uk/ScrumPy

The FRIM team