



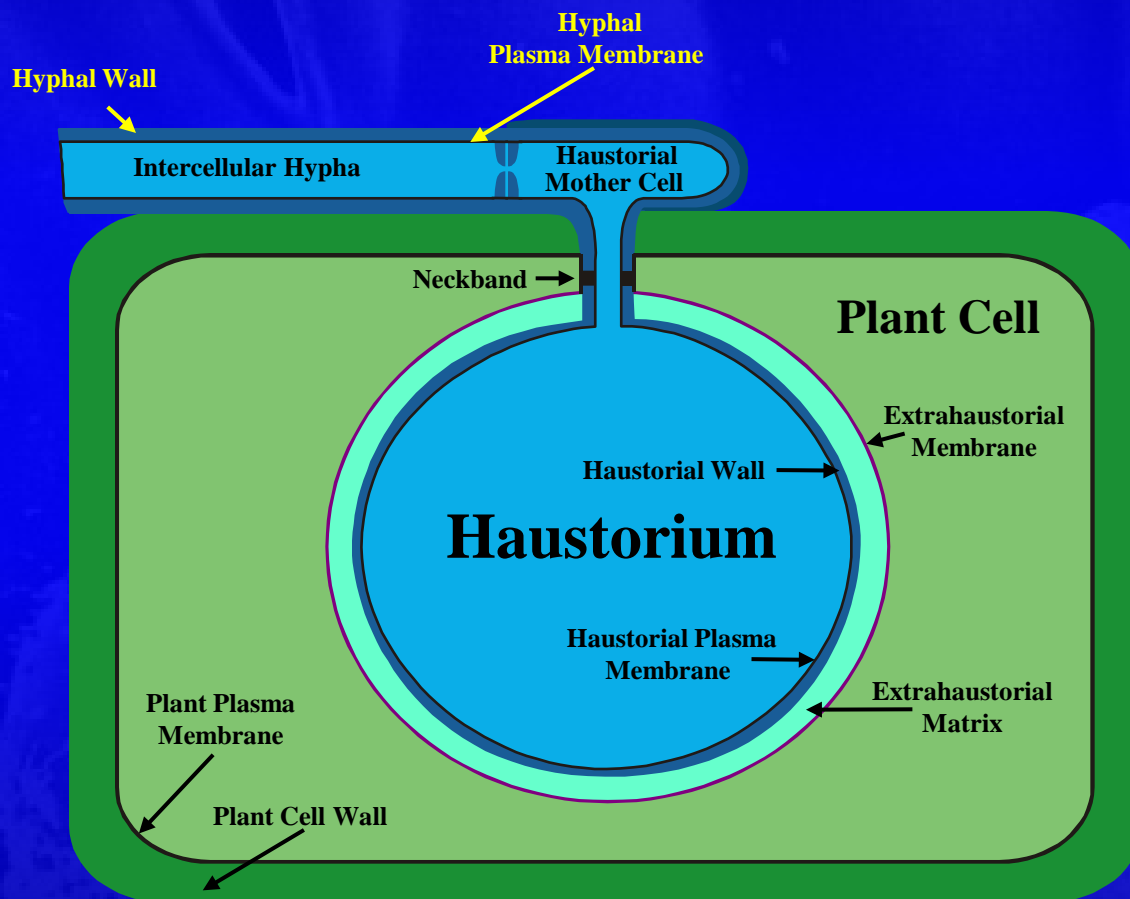
How Sweet is Parasitic Life?

Nutrient Uptake in Rust Fungi

Uromyces fabae on *Vicia faba*



A Close-Up of the Interface



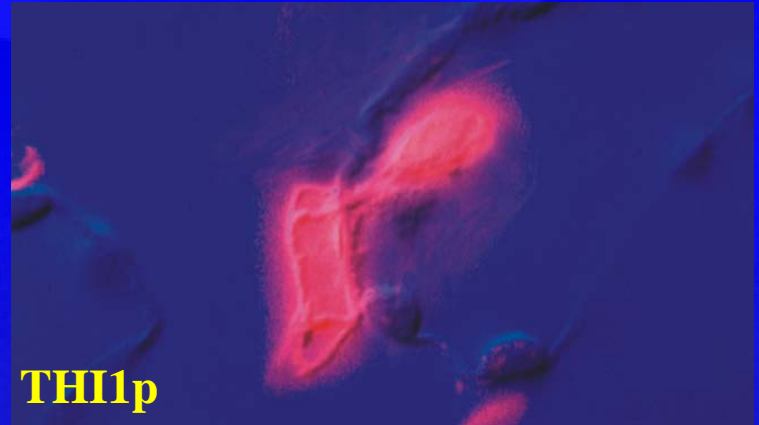
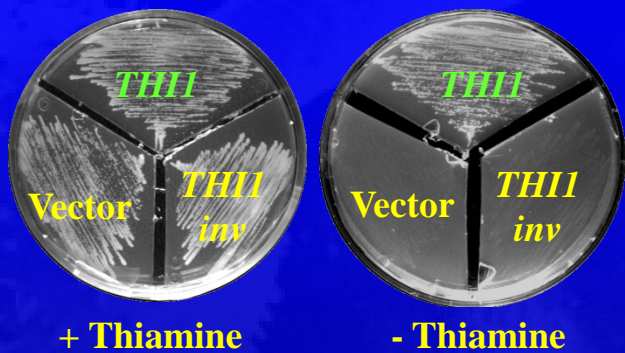
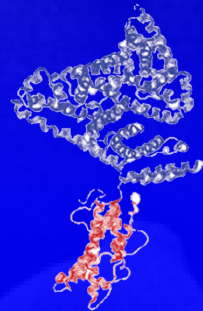
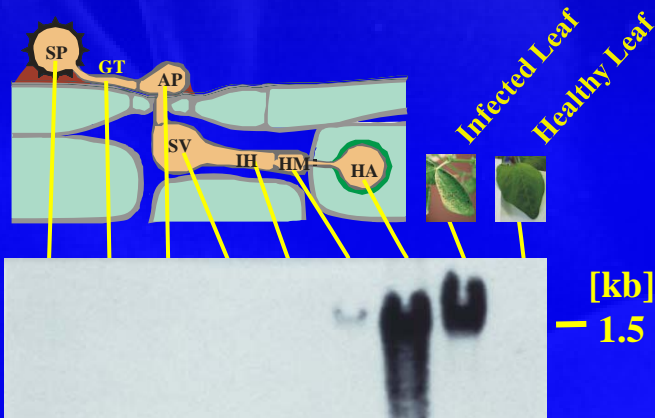
Coffey et al. 1972. *Can. J. Bot.* 50:231-240



mRNA Abundance in Haustoria

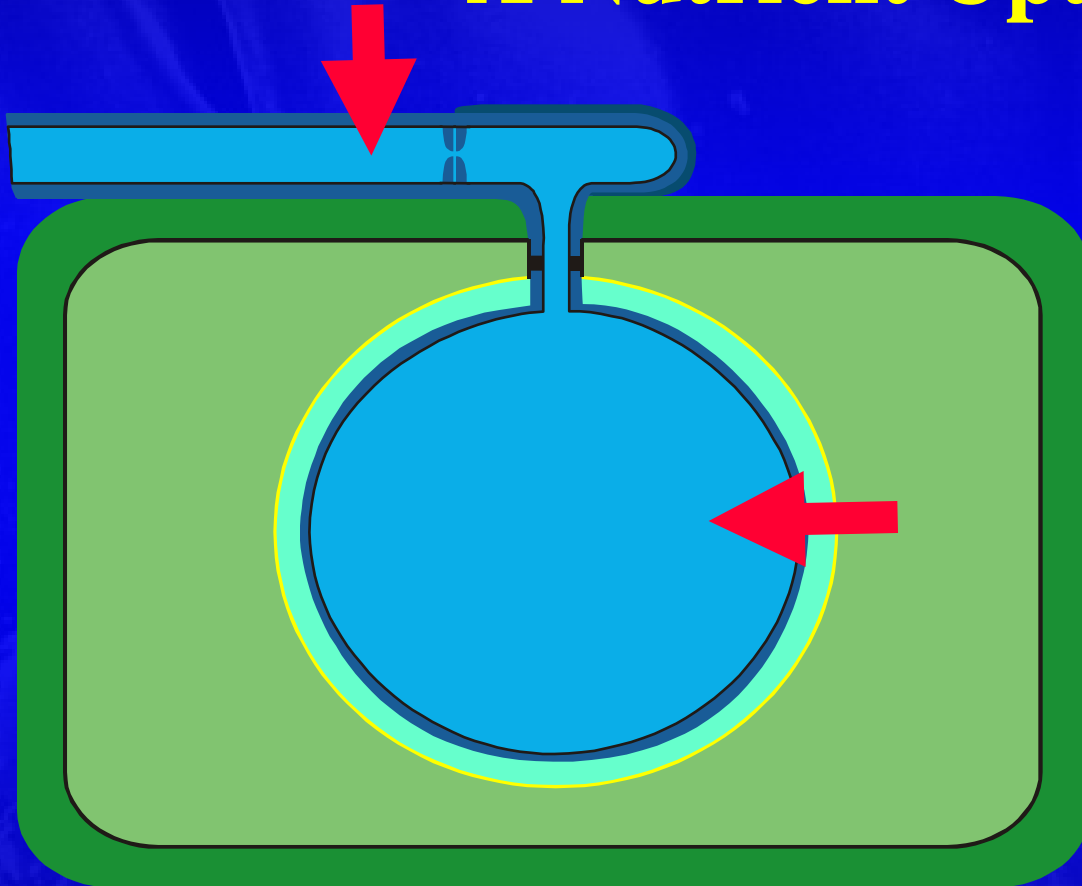
~ 5%	Vit B1 Synthesis.....	<i>THI1</i>
		<i>THI2</i>
~ 1%	H ⁺ - ATPase.....	<i>PMA1</i>
~ 3%	Amino Acid Uptake.....	<i>AAT1</i>
		<i>AAT2</i>
		<i>AAT3</i>
~ 1%	Hexose Uptake.....	<i>HXT1</i>
< 1%	Invertase.....	<i>INV1</i>
~ 1%	Mannitol Metabolism.....	<i>MAD1</i>

The Tool Box



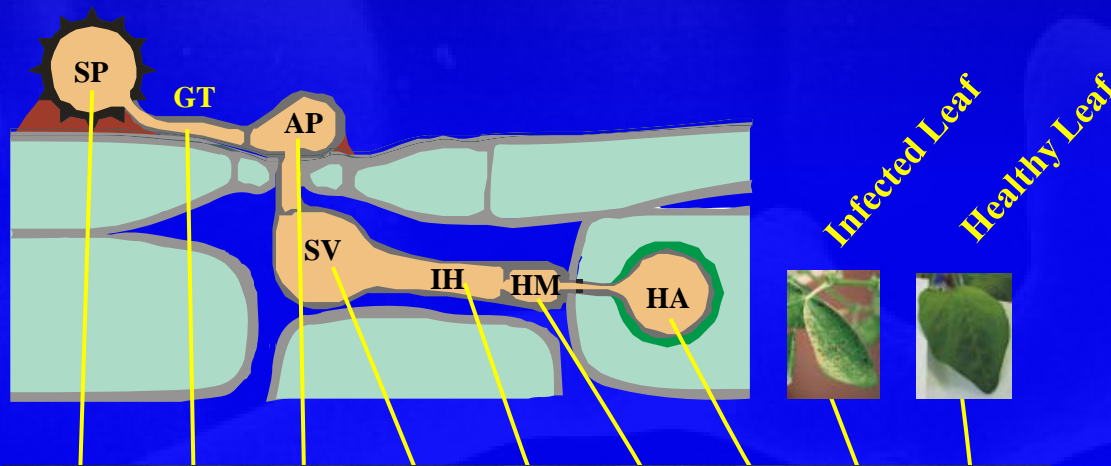
Rust Haustoria are Small Power Plants

The Haustorium: A Nutrient Uptake Device?



**Amino Acid
Uptake**

Transporter Expression



PMA1

AAT1

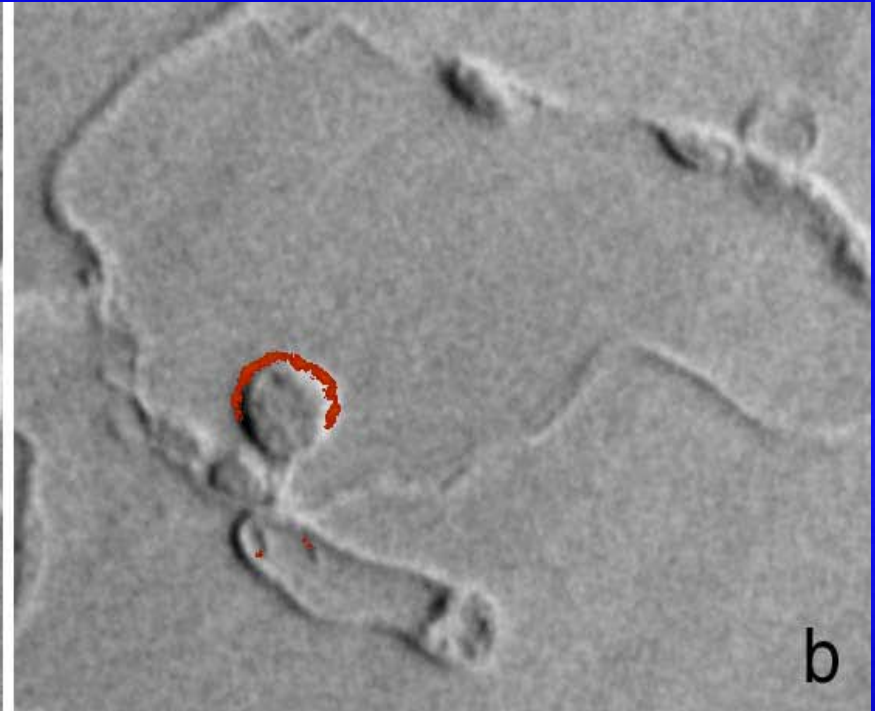
AAT2

AAT3

Localization of AAT2p



Monokaryotic



Dikaryotic

Haustoria

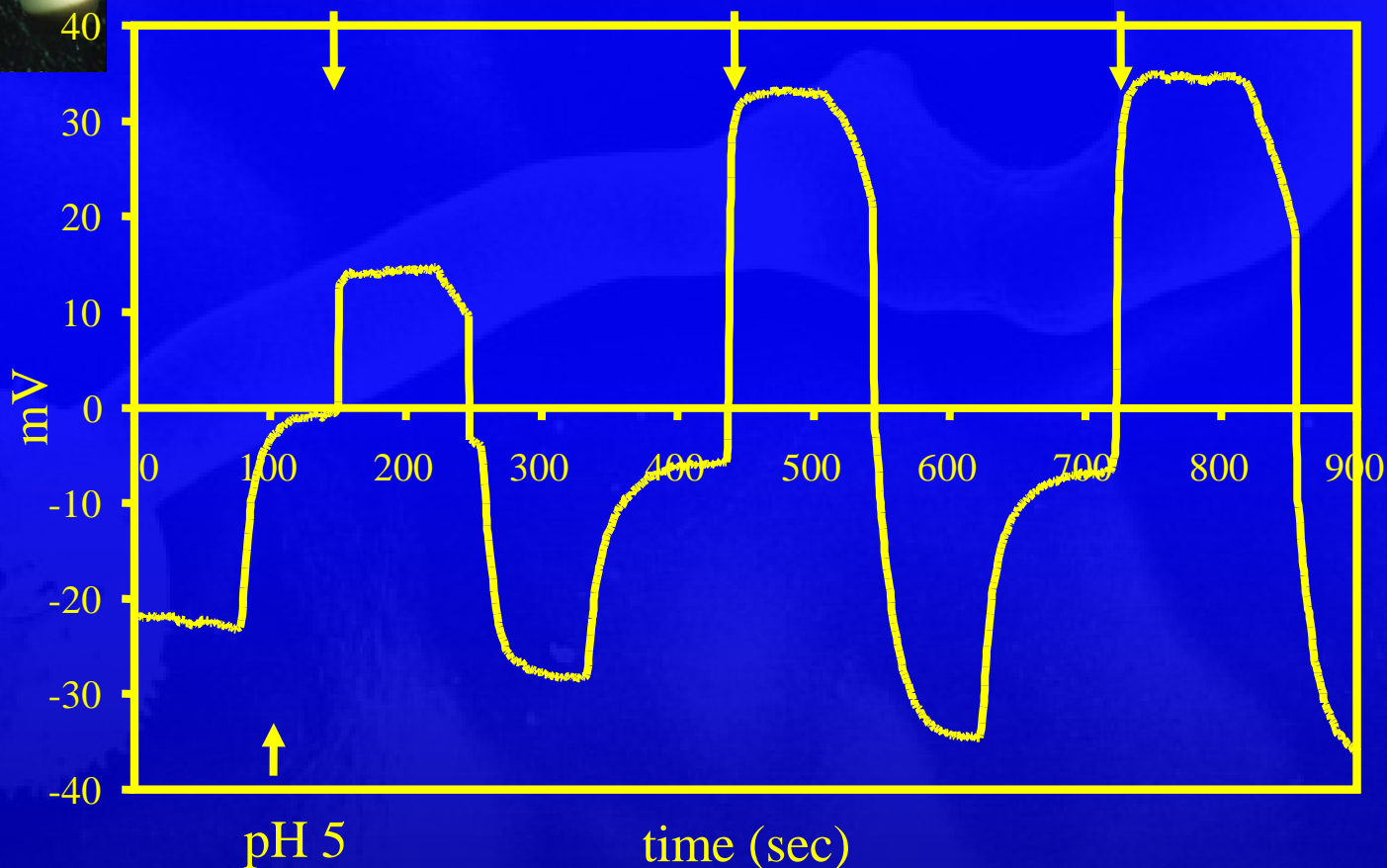
Analysis of AAT1p in *X. laevis* Oocytes



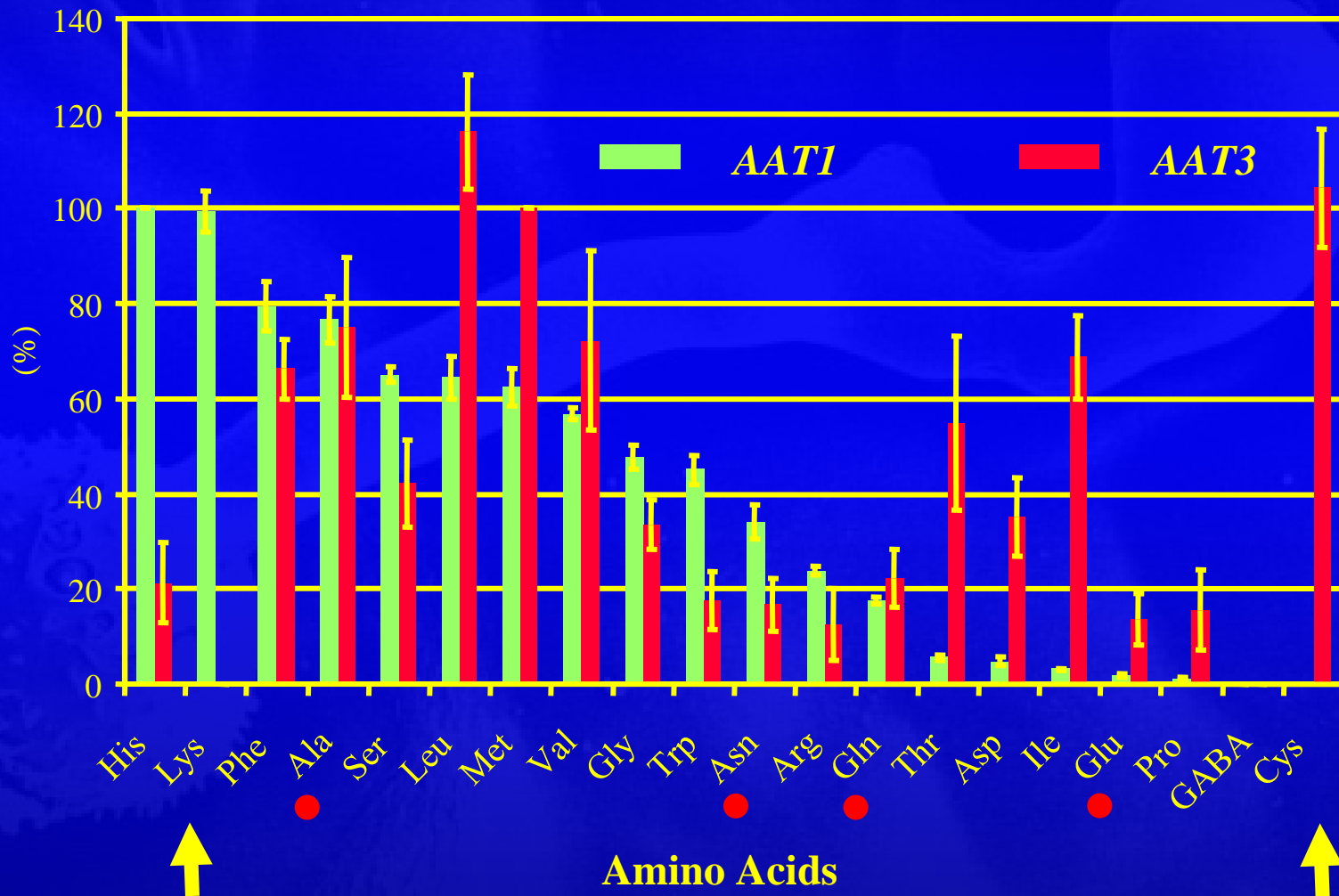
L-Arginine

L-Lysine

L-Histidine

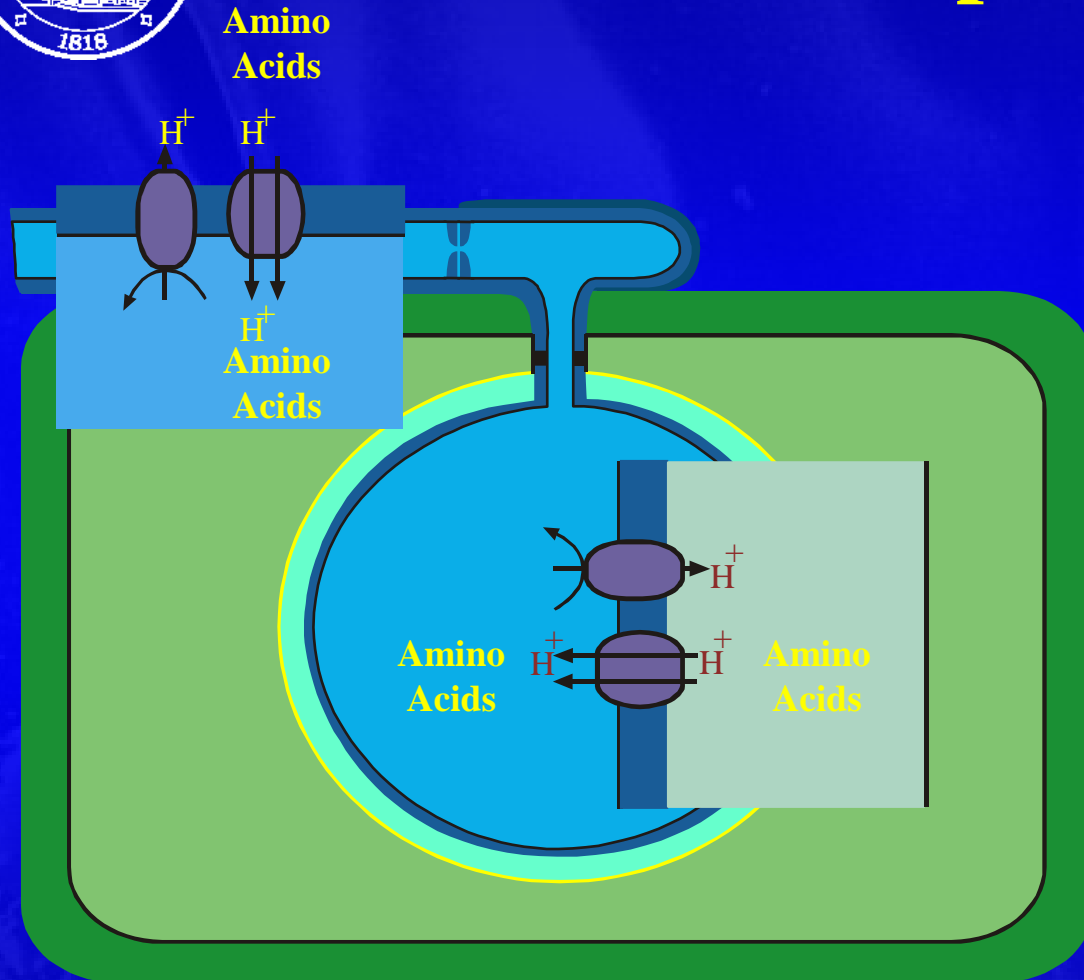


Comparison of AAT1p and AAT3p Specificity



Amino Acid Uptake

in *U. fabae*



Different Transporters

Different Specificities

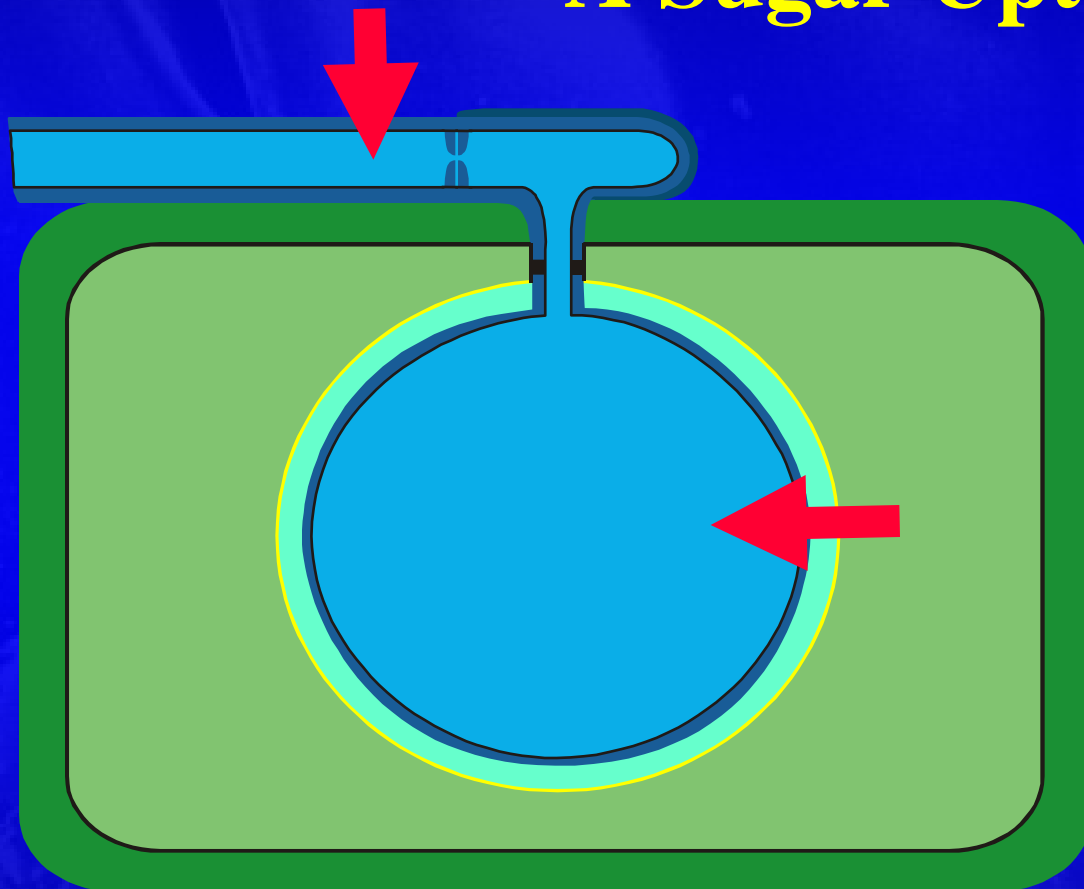
“Scarce” Amino Acids

Uptake in Haustoria

**Uptake in Intercellular
Hyphae**

AA Uptake is not Haustorium Specific

The Haustorium: A Sugar Uptake Device?

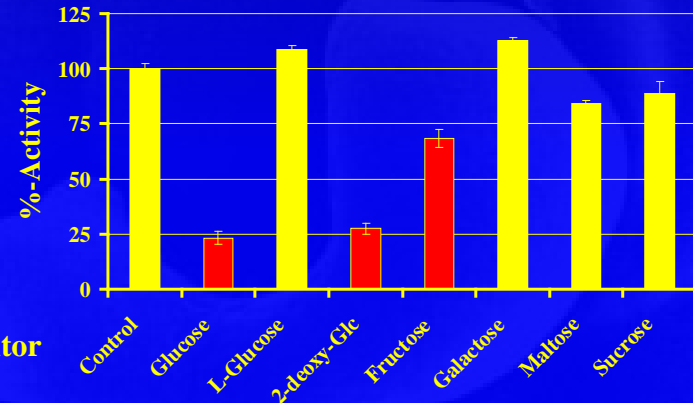
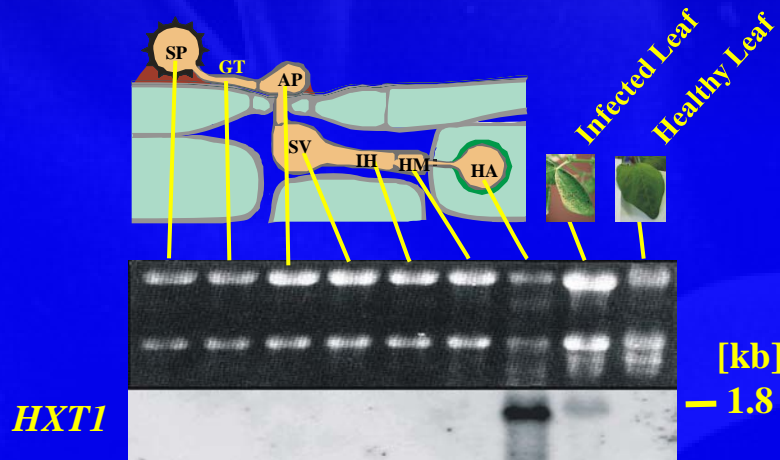


HXT1: 3,138 bp
ORF: 1,569 bp
HXT1p: 522 aa
56.8 kDa

**Major Facilitator
Superfamily**

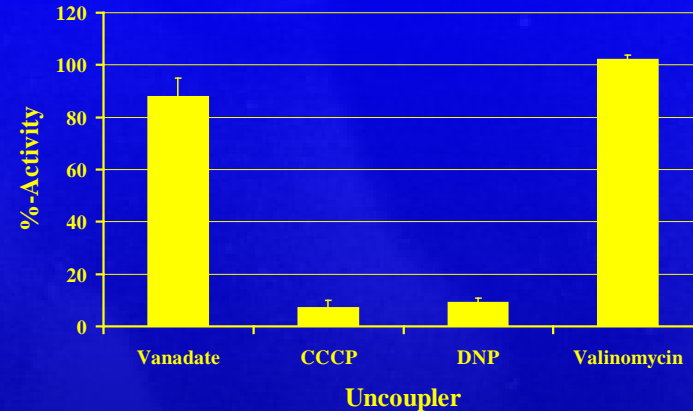
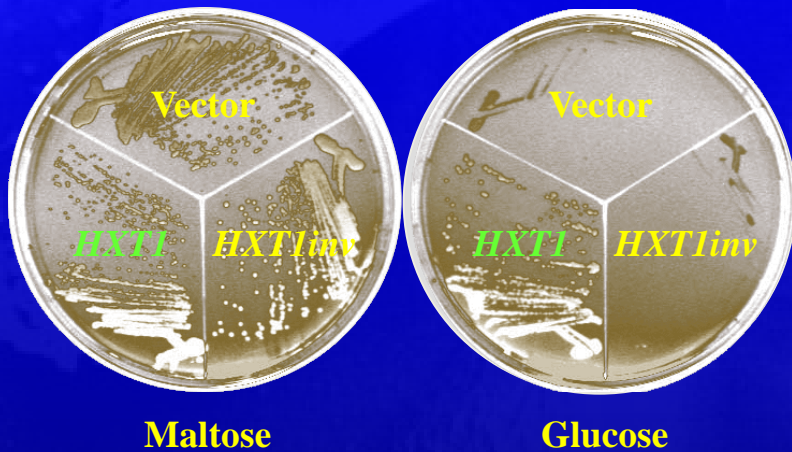
TC 2.A.1.1

Characterization of HXT1p

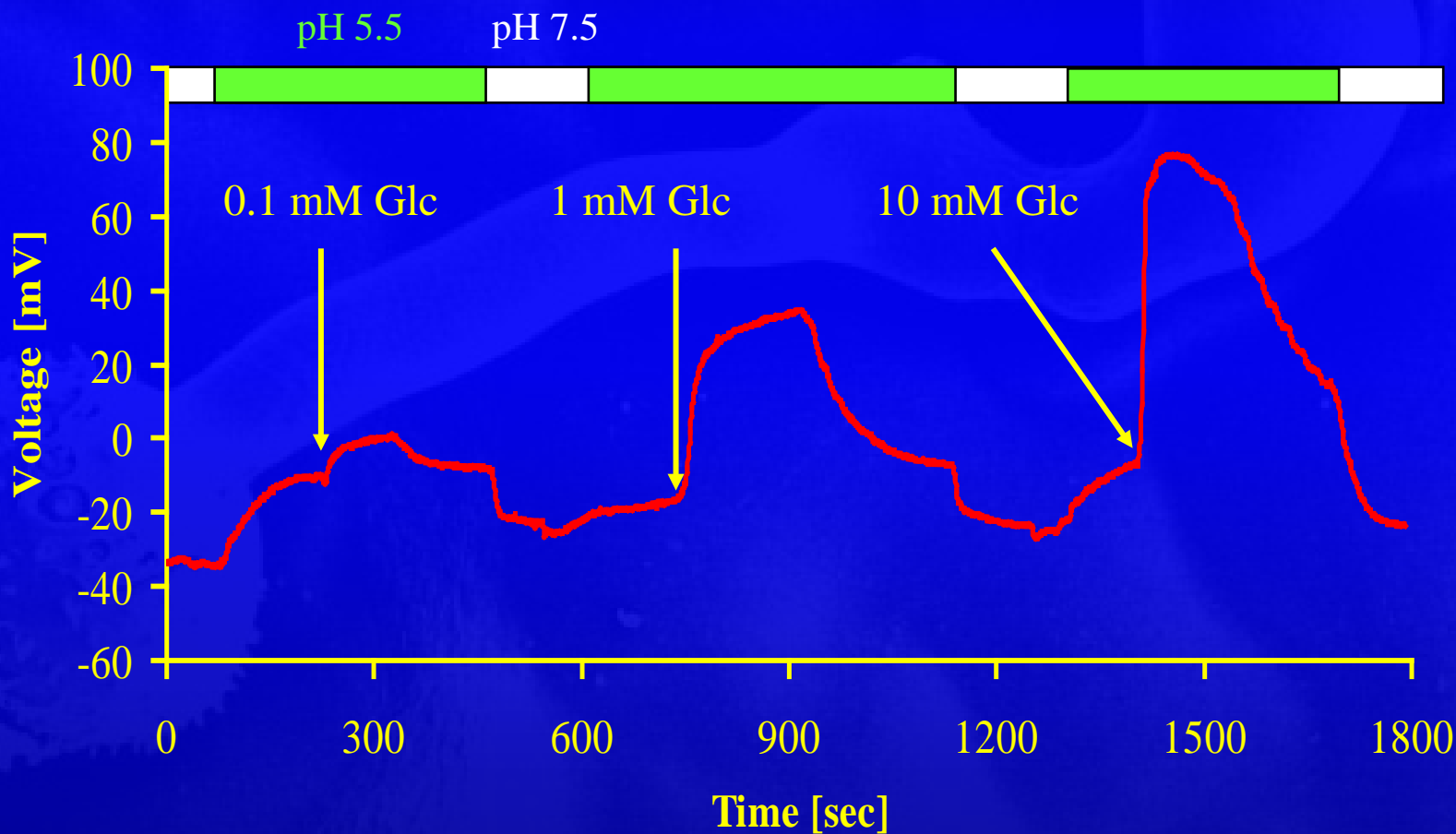


Competitor

D-Glucose: $V_{max} = 80 \text{ nmol}/(\text{min} \cdot \text{mg protein})$, $K_M = 0.35 \text{ mM}$
 D-Fructose: $V_{max} = 55 \text{ nmol}/(\text{min} \cdot \text{mg protein})$, $K_M = 1.00 \text{ mM}$

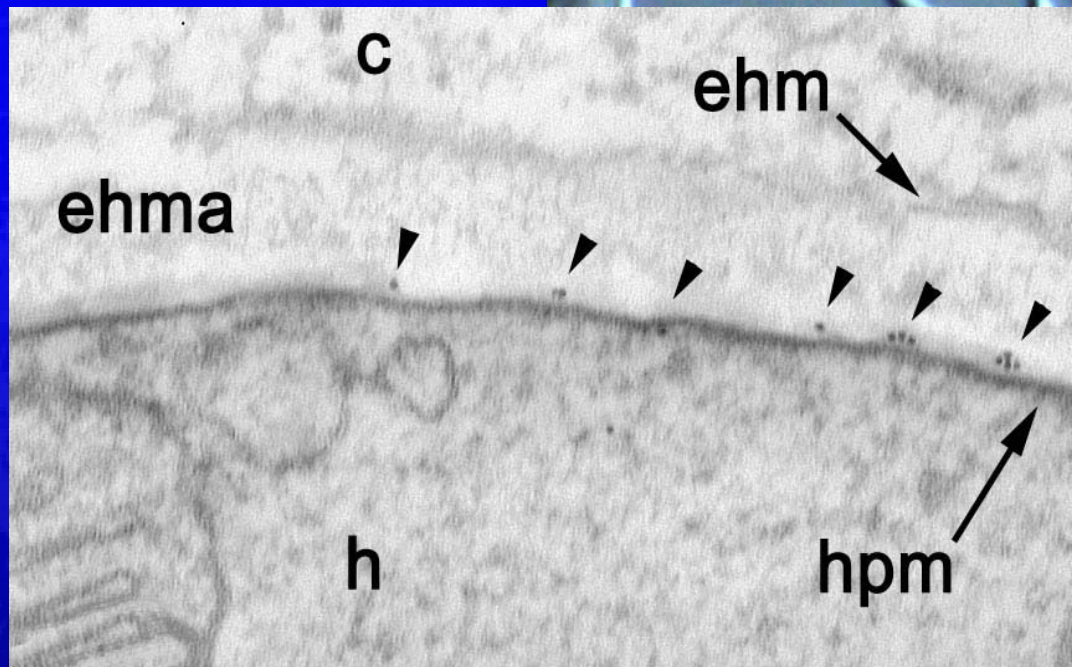
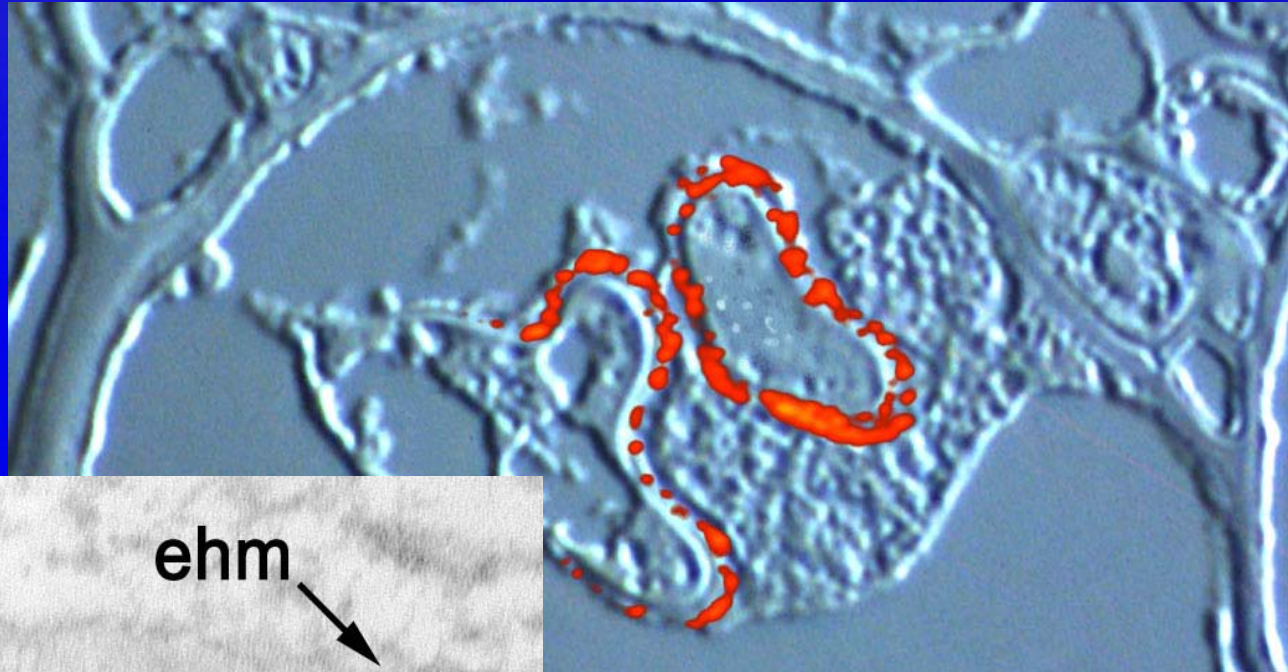


HXT1p Analysis in *X. laevis* Oocytes



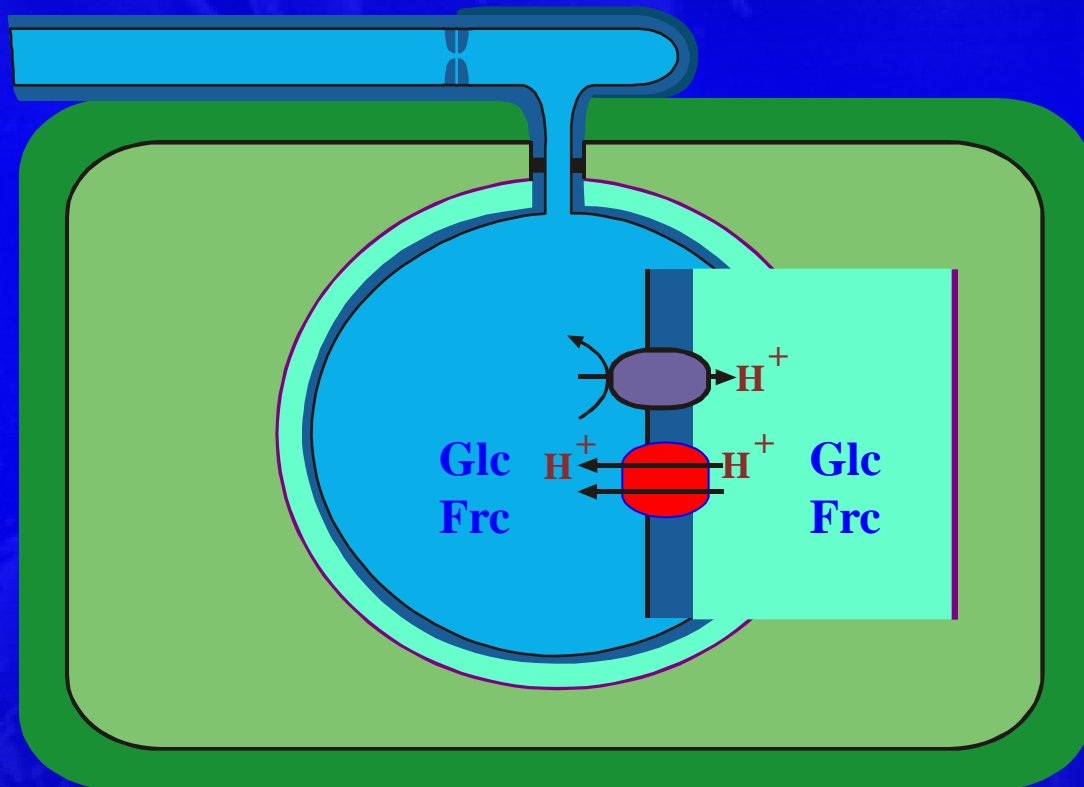
Immunocytochemistry of HXT1p

Localization
using S651p



Carbohydrate Uptake

in *U. fabae*



**Only One Transporter
HXT1p**

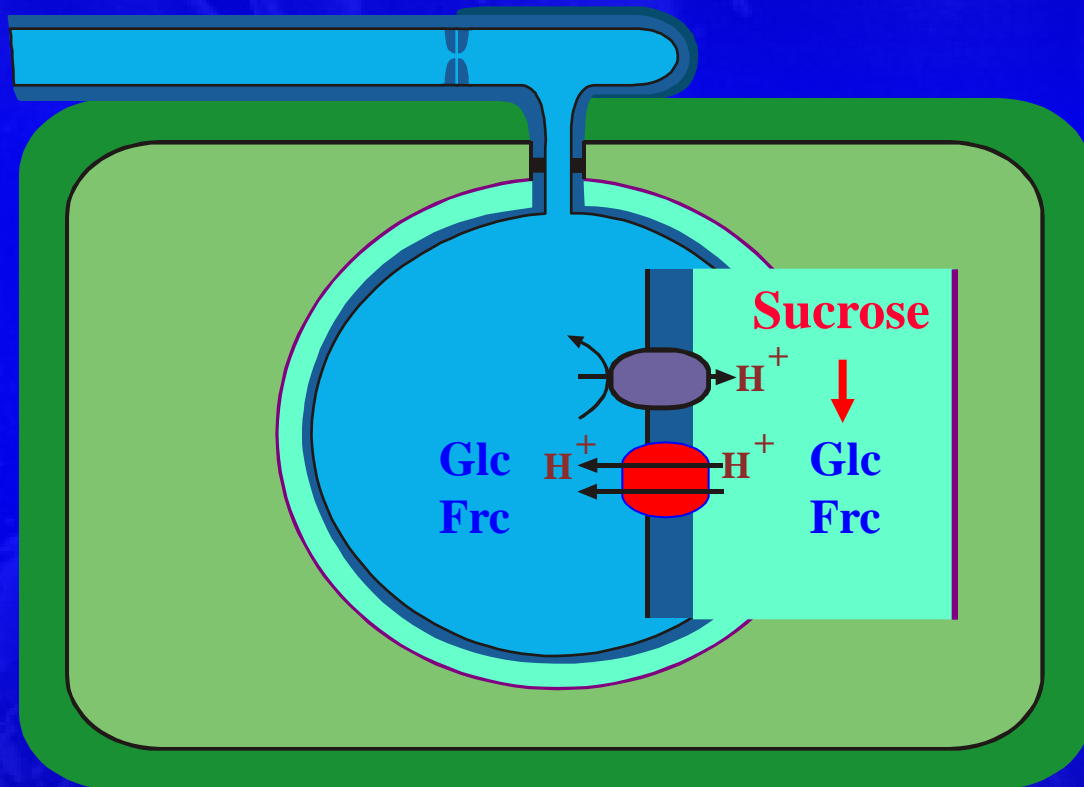
**Exclusively
Through Haustoria**

**Specificity for
D-Glucose
D-Fructose**

PMF-Driven

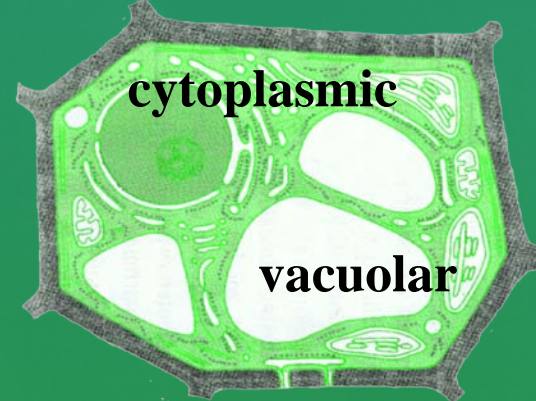
Rust Haustoria are Sugar Uptake Devices

Where Do the Sweets Come from ?



**Fungal
Invertase
?**

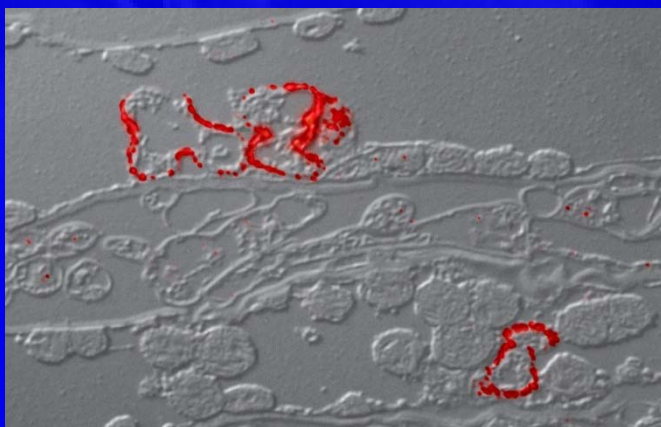
**Plant
Invertases**
cell wall bound



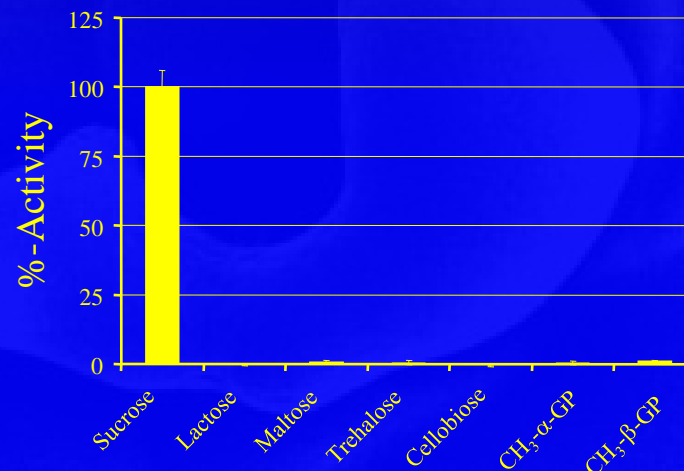
INVERTASE

Characterization of INV1p

ORF: 2,265 bp INV1p: 754 aa (25 aa SS)

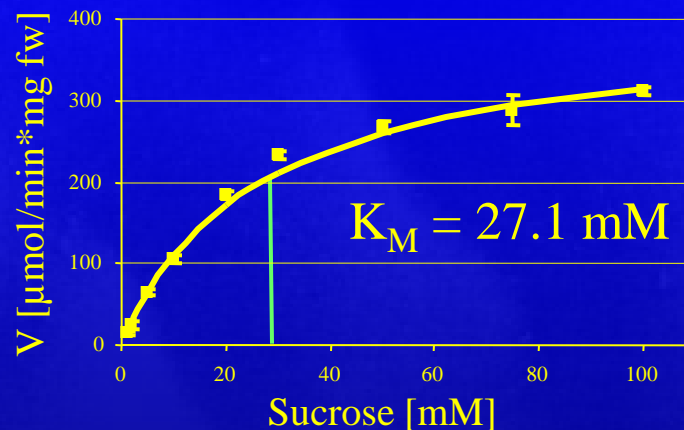


Localization using S698p

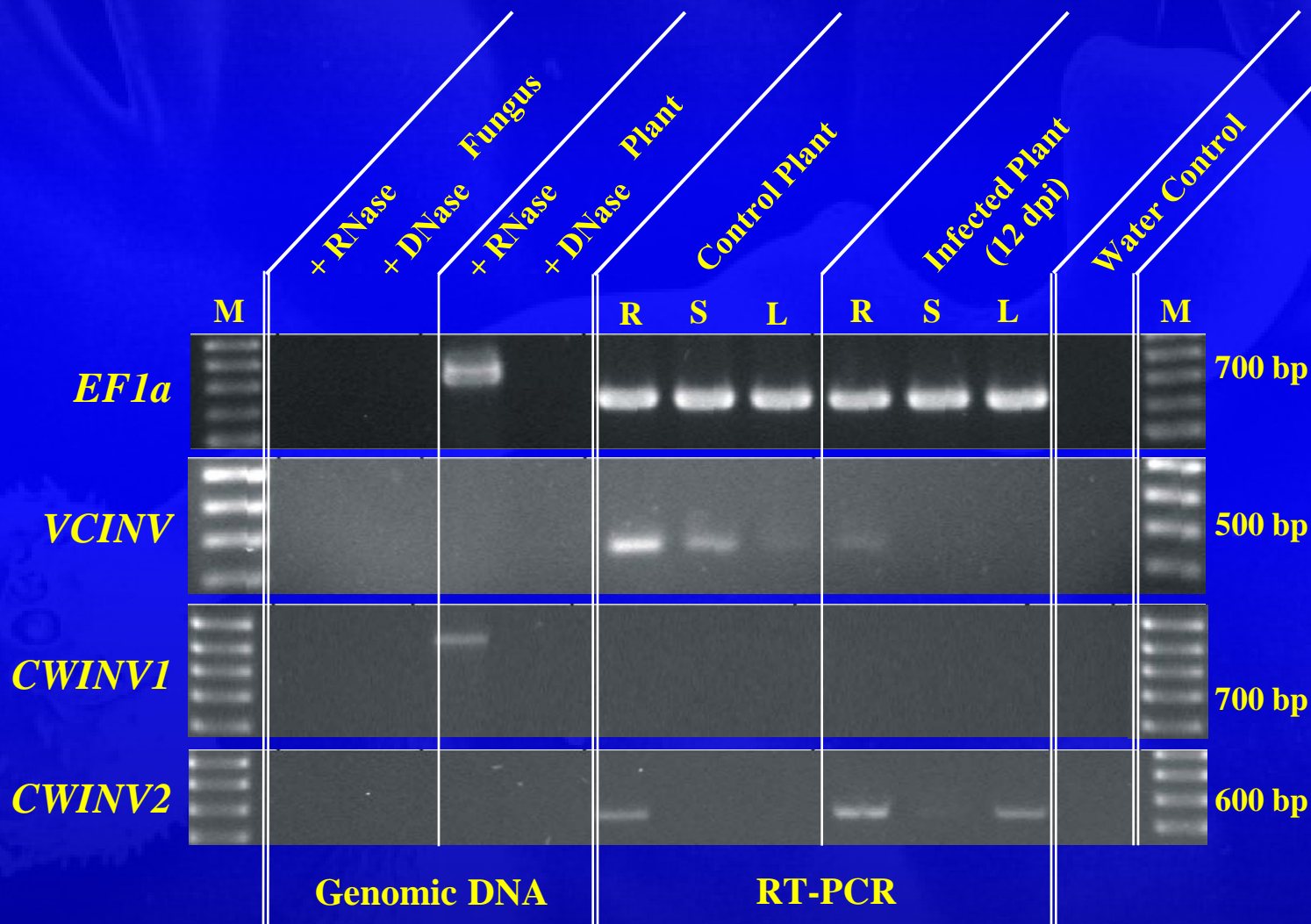


Glucose

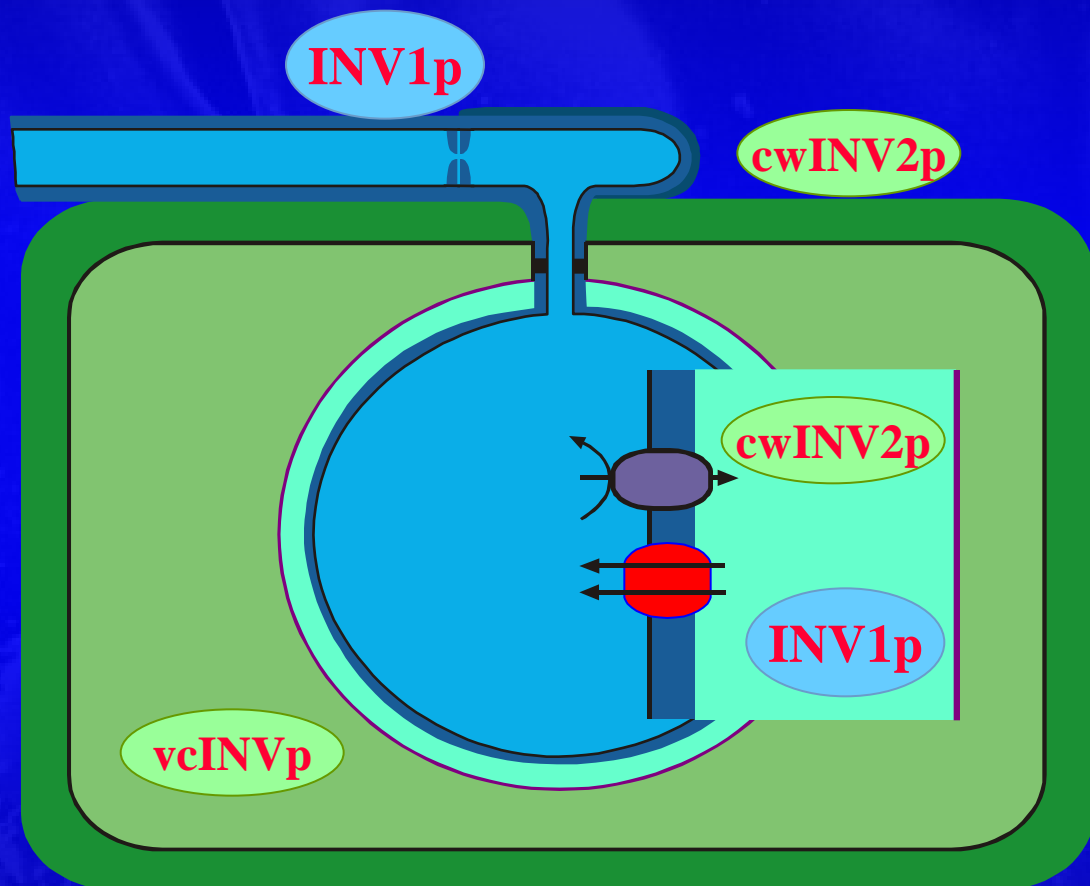
Sucrose



The Role of Plant Invertases



Carbohydrate Mobilization in *U. fabae*



**Fungal and plant
invertases are involved
in sucrose cleavage**

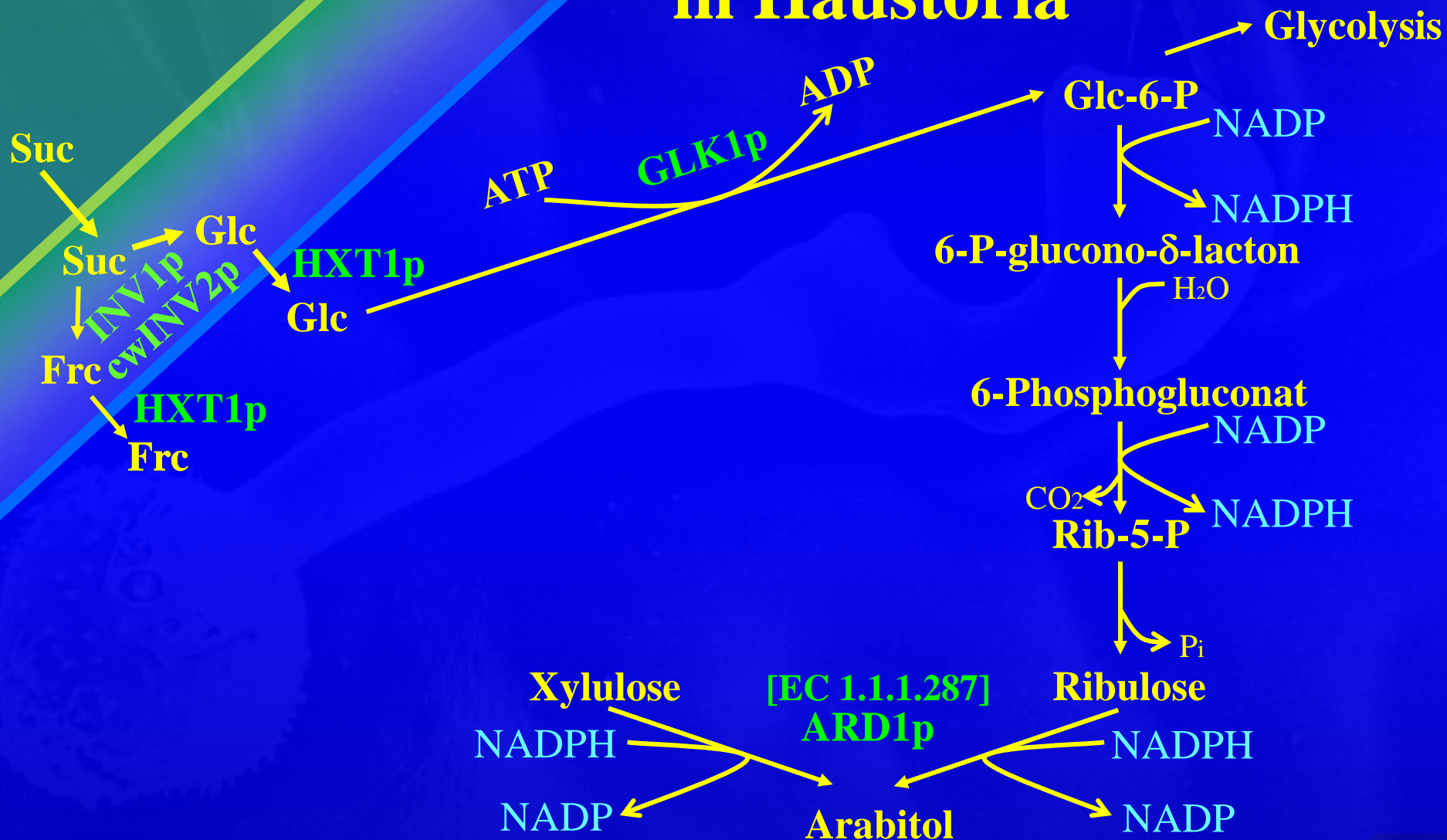
**Plant invertases are
affected differentially**

**Effects on remote
plant organs**

Conversion of Source Tissue to Sink Tissue

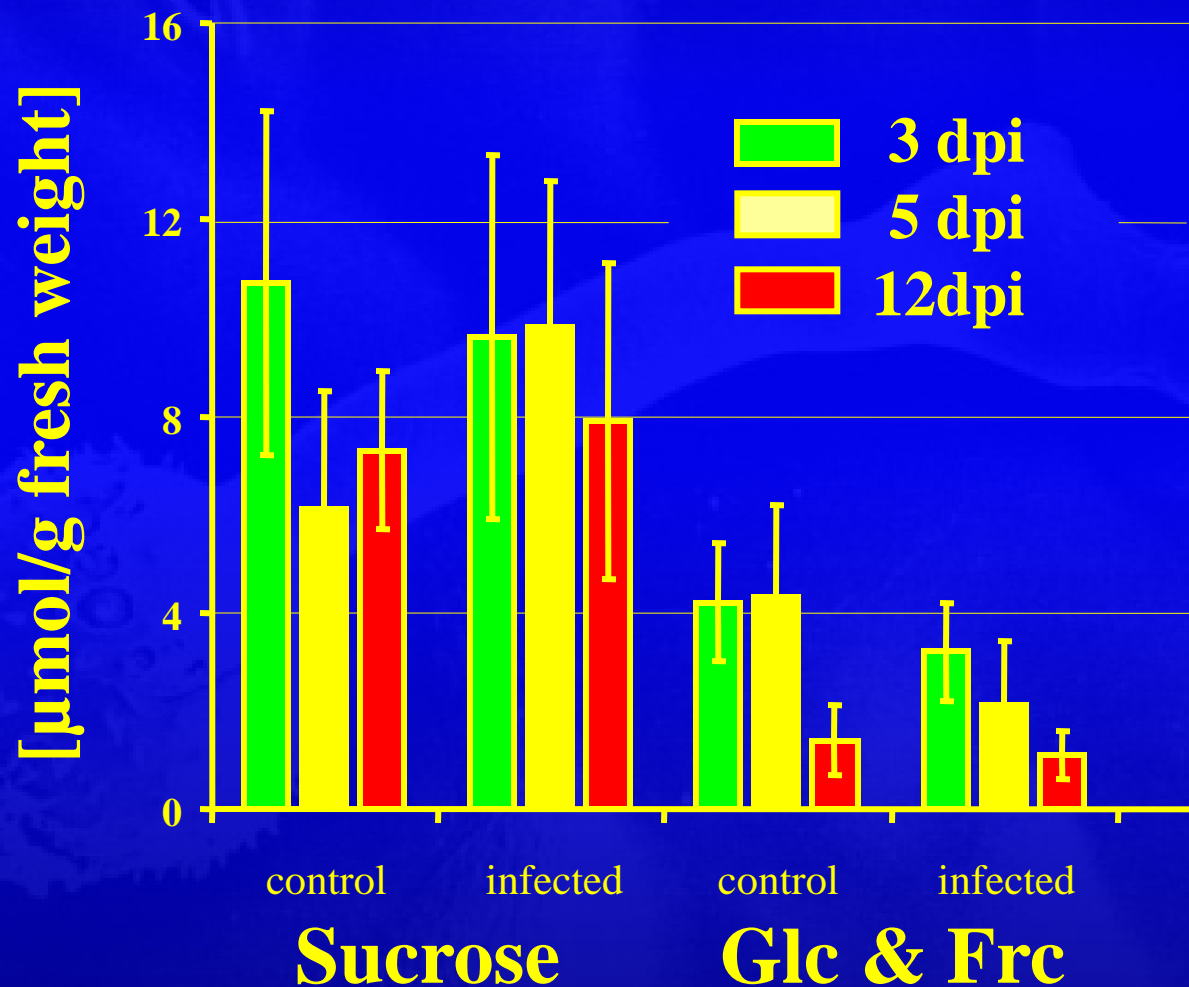


Carbon Metabolism in Haustoria



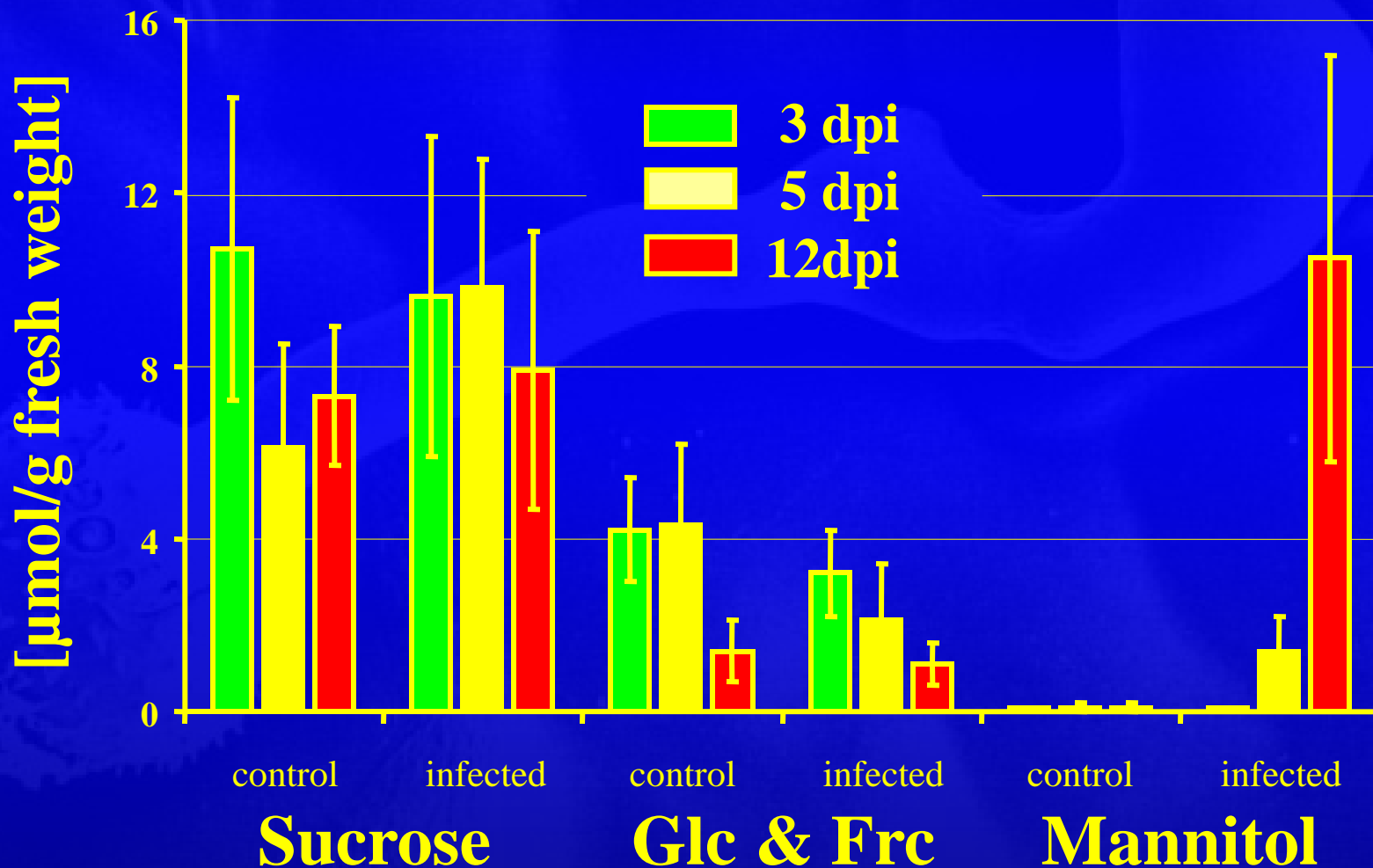
Fructose Metabolism

in *U. fabae*



Fructose Metabolism

in *U. fabae*





What's Interesting About Mannitol

Usually Not Present in Plants





Uromyces fabae

Mannitol Dehydrogenase 1

Homology to *Agaricus bisporus* Mannitol Dehydrogenase



Substrates Mannitol 78 mM

Fructose 804 mM

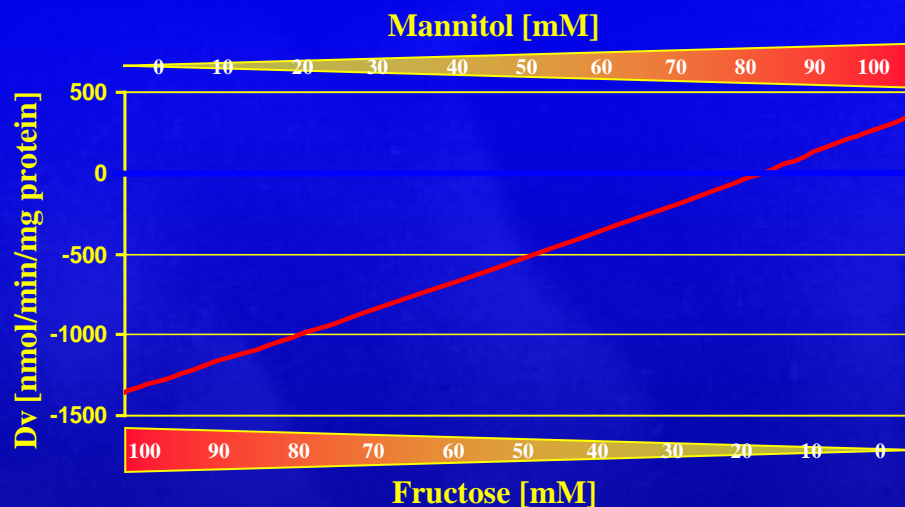
Co-Factors NADP 34 μM

NADPH 38 μM

Alt. Subst. Sorbitol

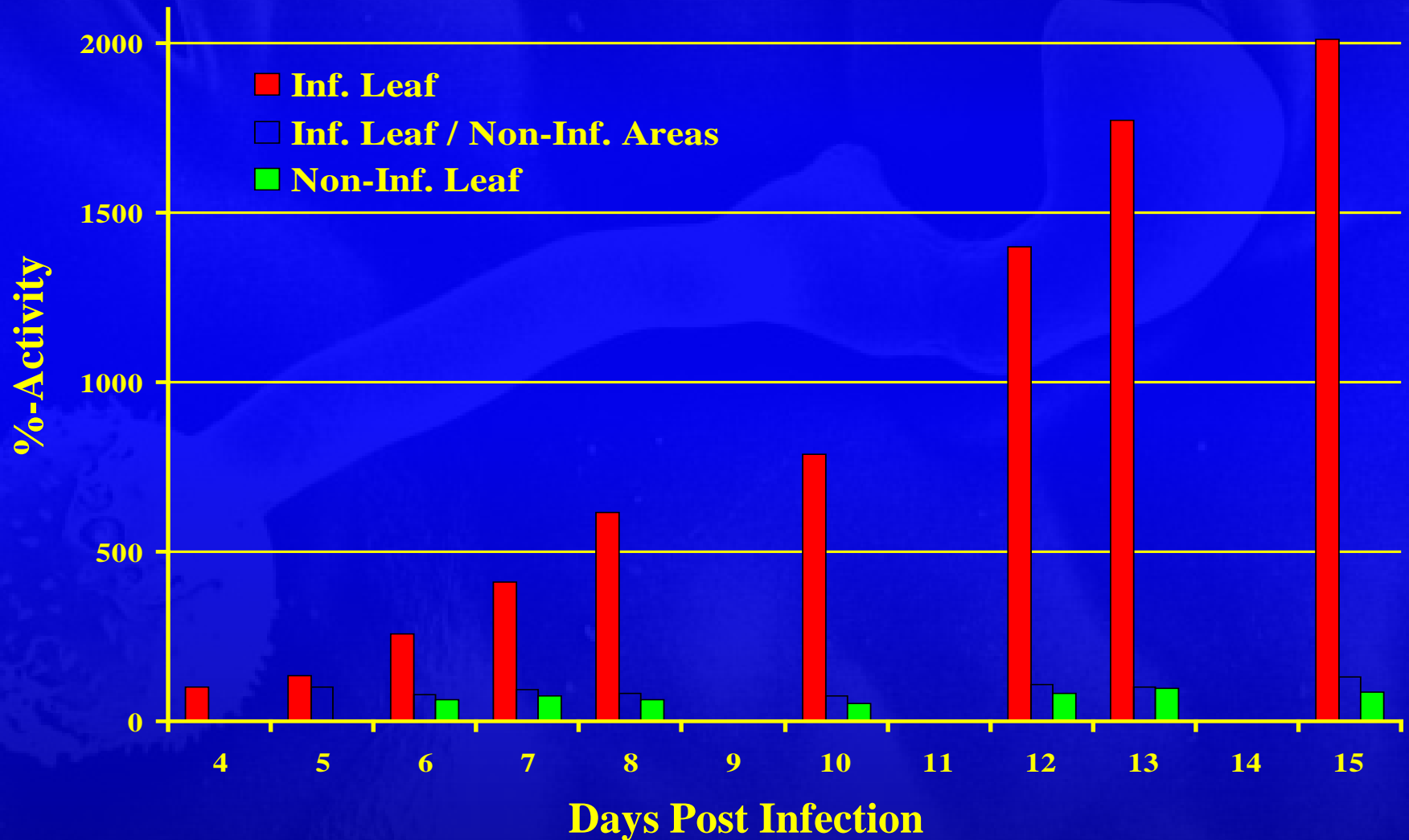
Class. EC 1.1.1.138

Equilibrium Constant: $6 \times 10^{-9} \text{ M}$

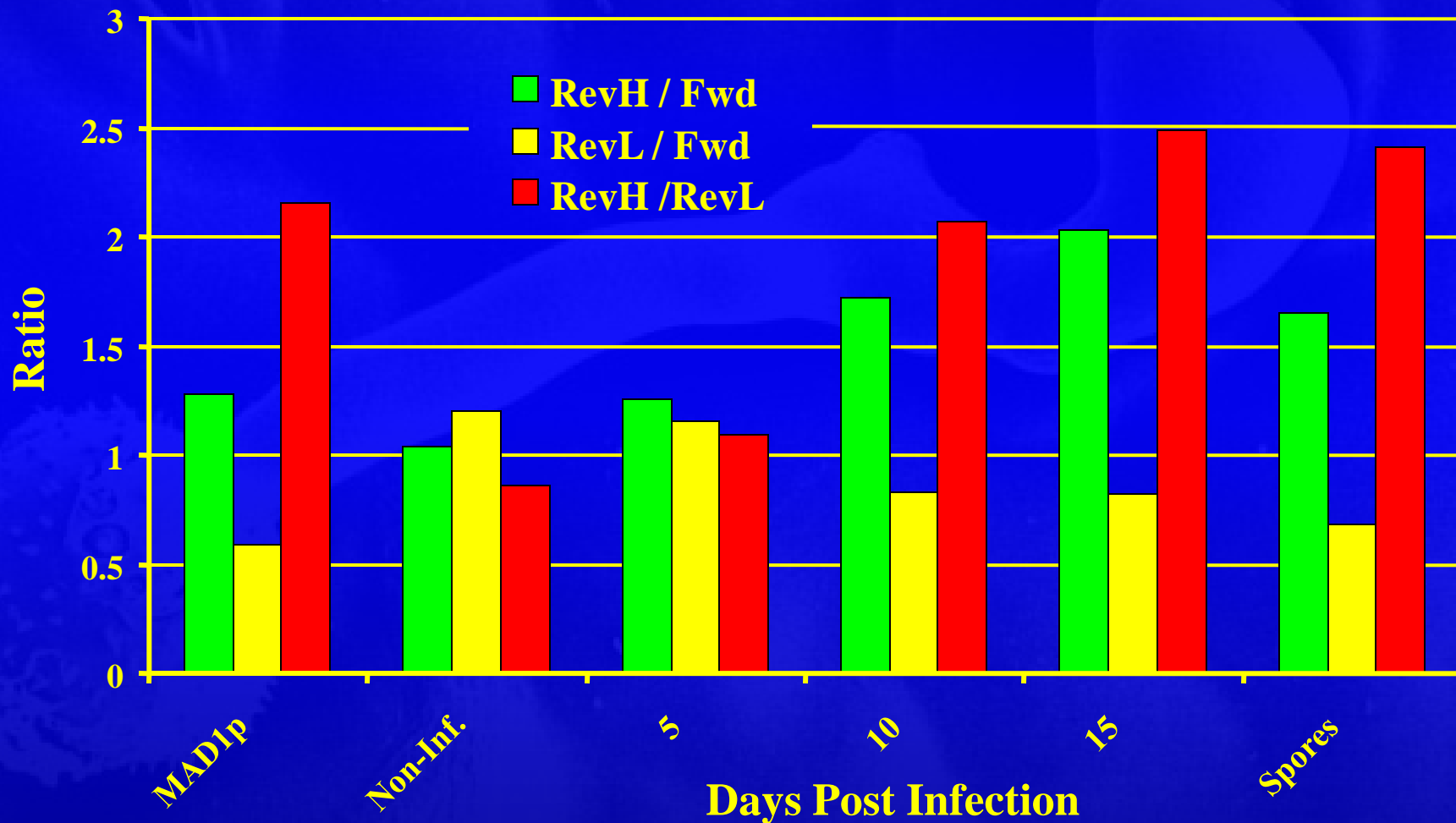




Mannitol Dehydrogenase Activity *in planta*



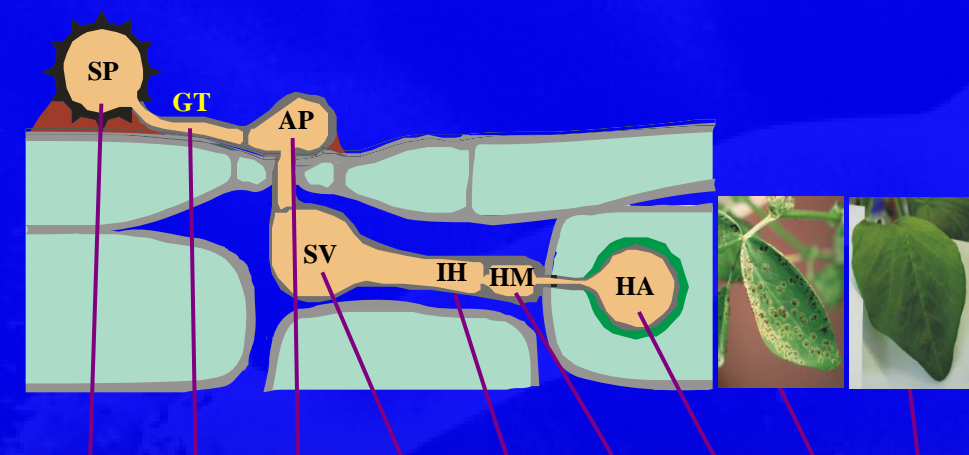
MAD1p, or Plant-MAD ?



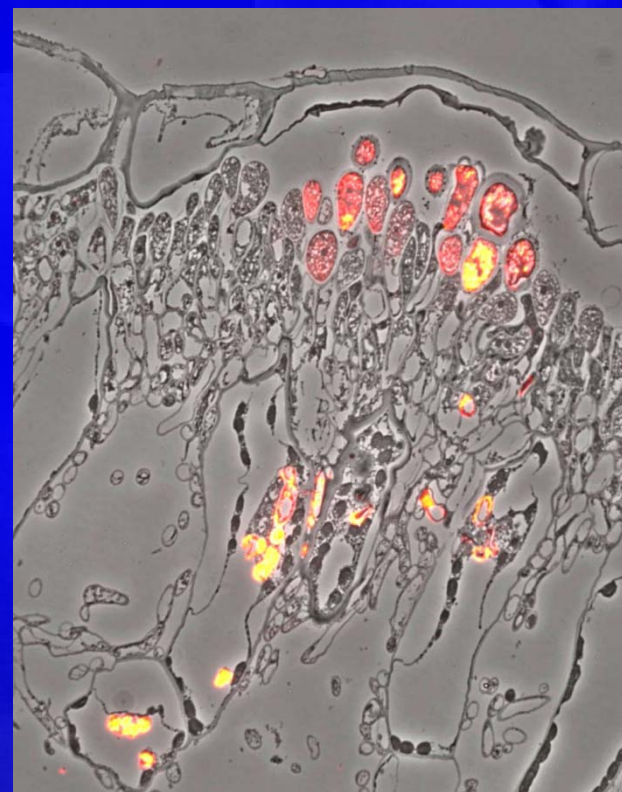
Localization of *MAD1* and MAD1p

MAD1

MAD1p

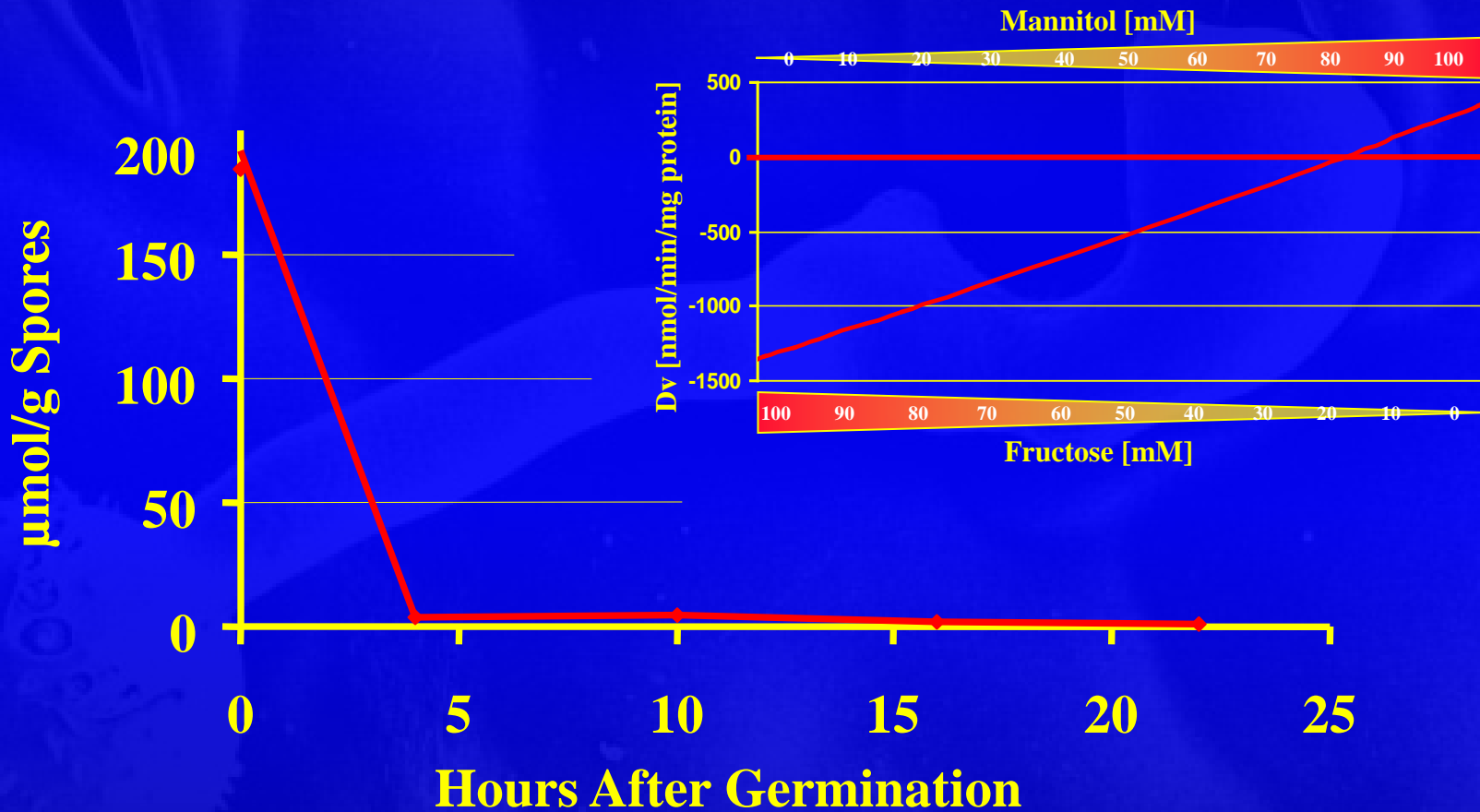


Expression pattern of *MAD1*



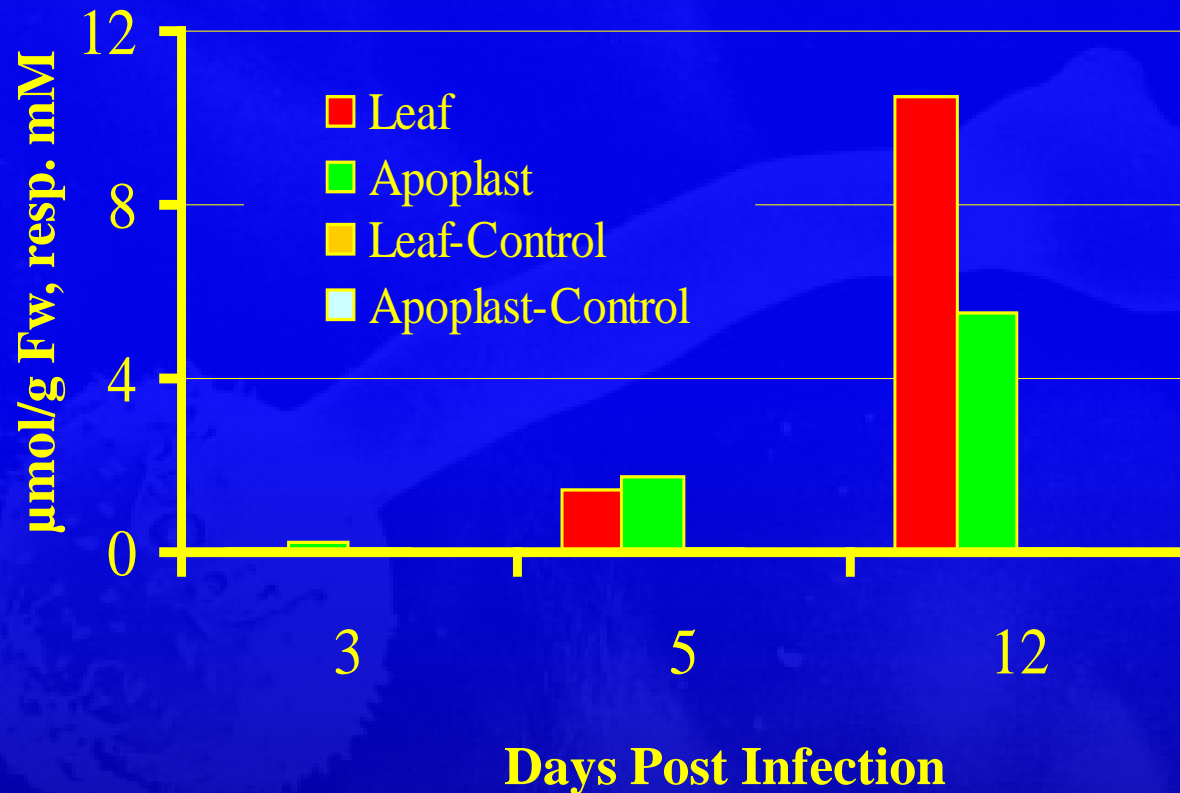
Localization of MAD1p
using S717p

Roles for Mannitol in *U. fabae*



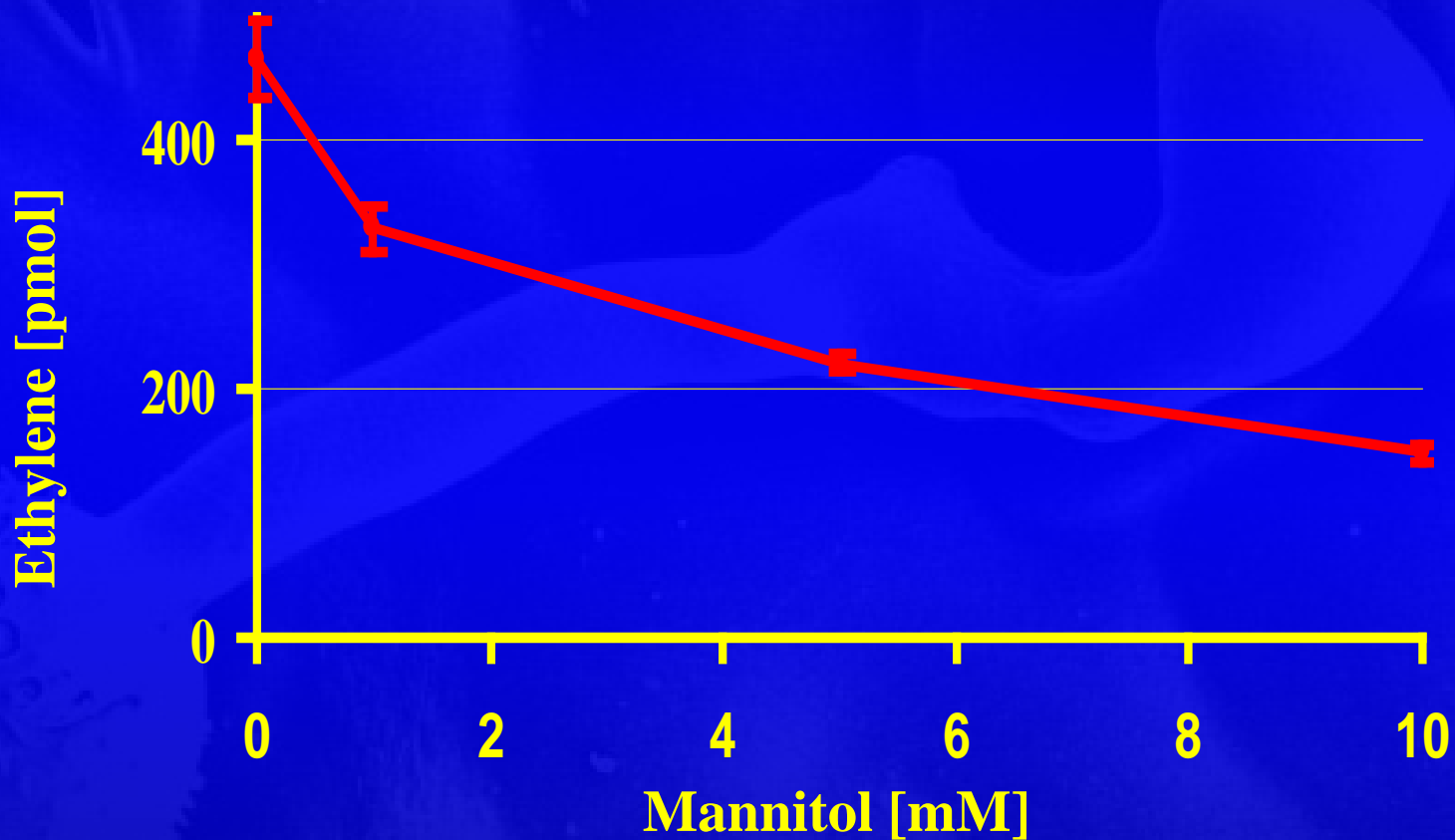
Role as Carbohydrate Storage Compound

Roles for Mannitol in *U. fabae*



Storage
Osmoprotection
Radical Scavenger
Conversion of Reducing Power

Roles for Mannitol in *U. fabae*



Role in the Suppression of Plant Defenses

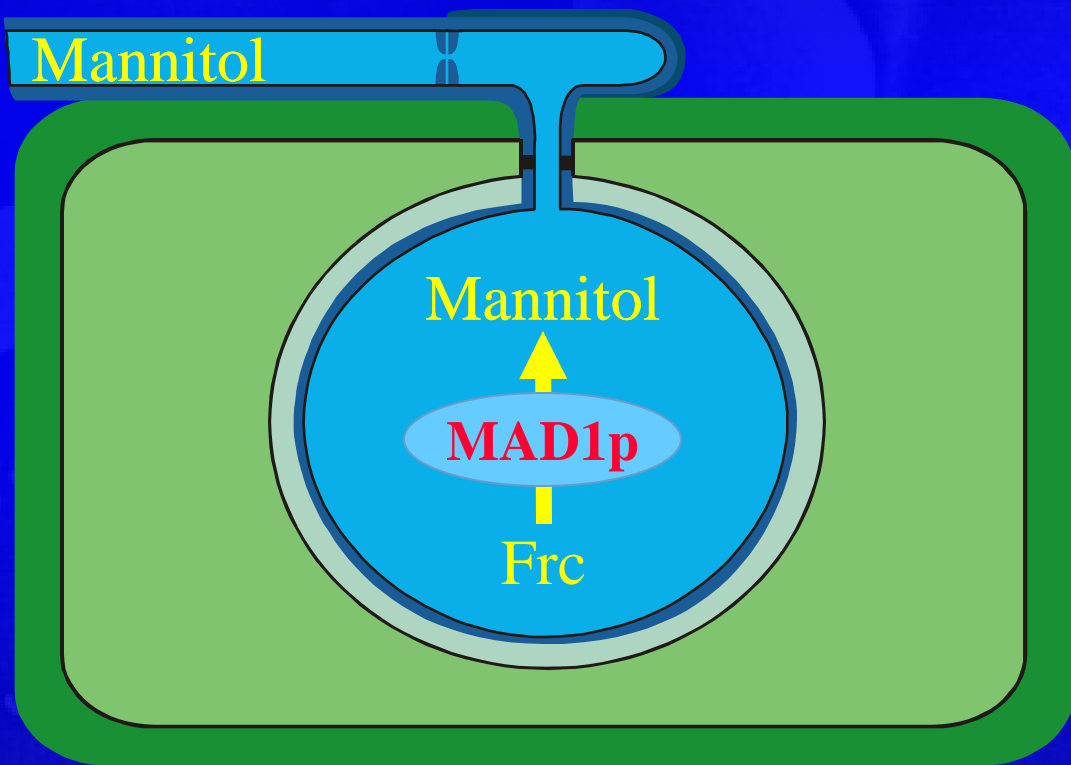
Mannitol in *U. fabae*

ROS
ROS
ROS
ROS
ROS

Frc ← MAD1p



Mannitol



- Produced in haustoria by MAD1p
- Diffusion in mycelium
- Secretion into apoplast
- Detoxification of ROS
- Deposition in spores
- Conversion into Frc by MAD1p in germinating spores



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