Glucosinolates and sulphur-rich cells

Distribution in roots of field-grown canola

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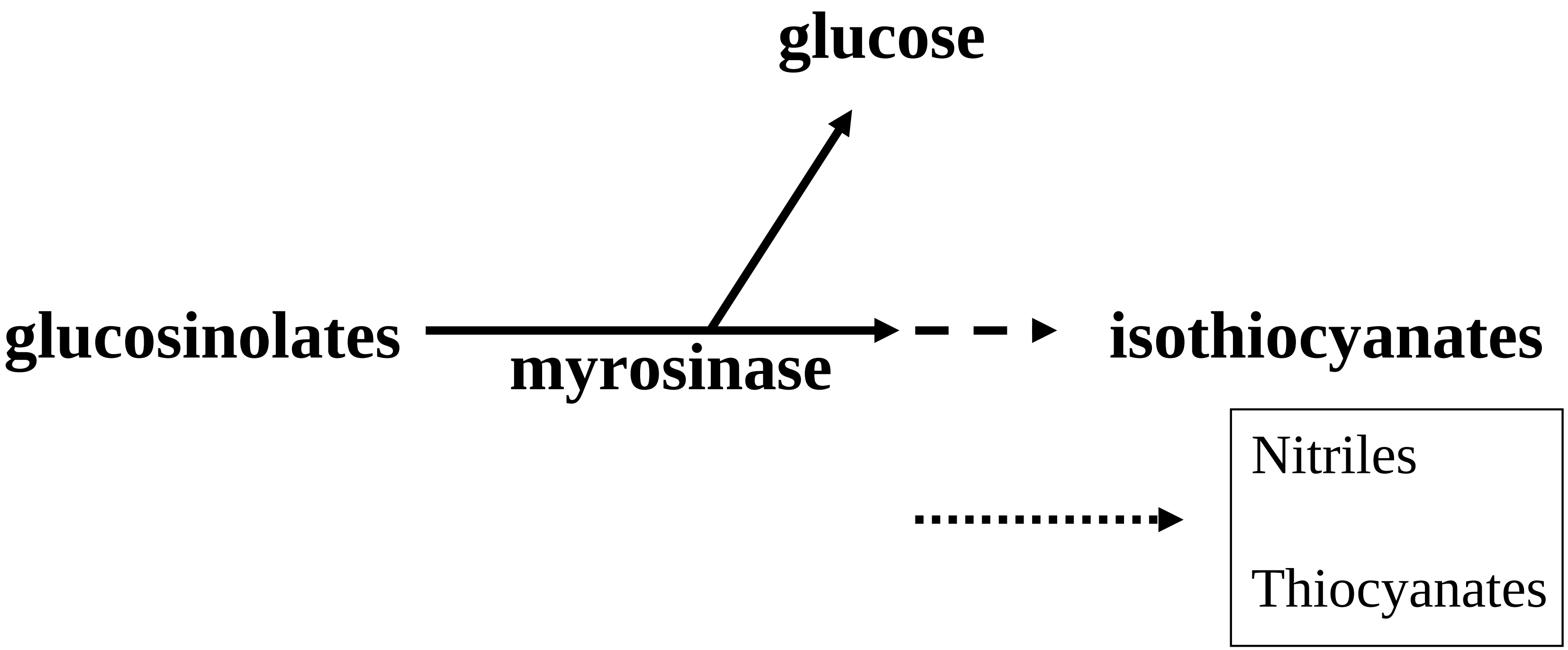
Biofumigation uses residues of glucosinolate-containing plants for pest and disease control

Much emphasis has been on incorporation of the above-ground plant material

Biofumigation harnesses the ‘mustard bomb” reaction

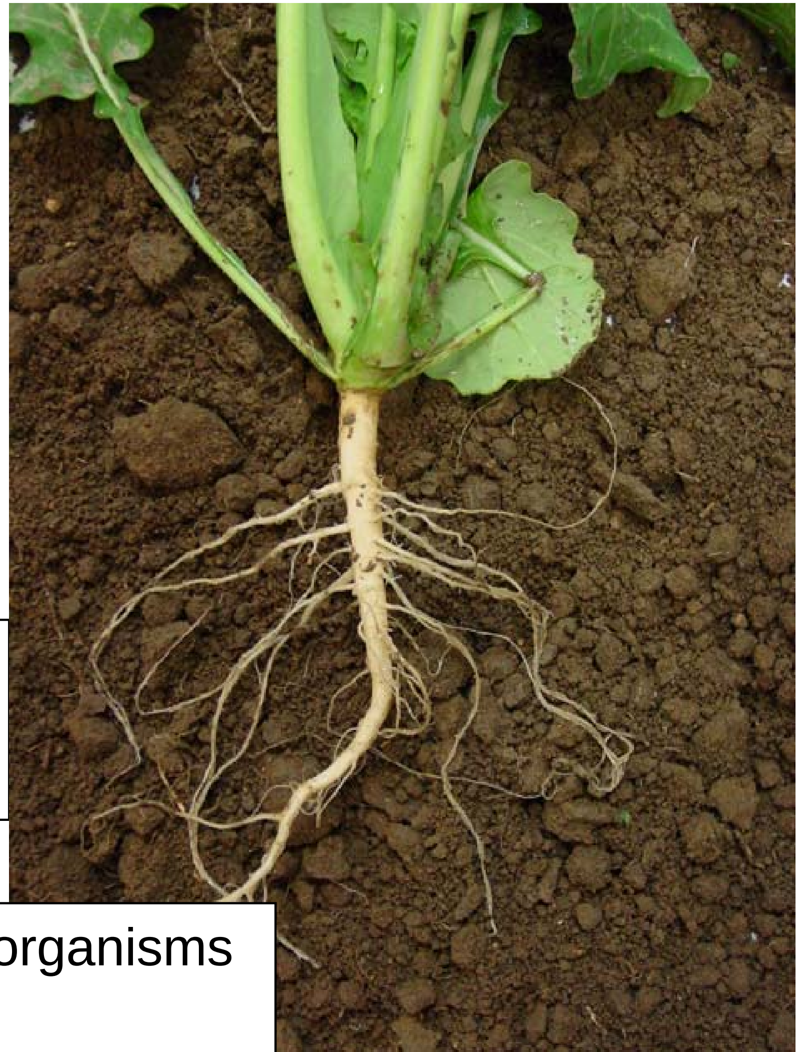


Why focus on *Brassica* roots?

On average roots have 4.5 X GSL concentrations in shoots

Van Dam *et al*. 2008

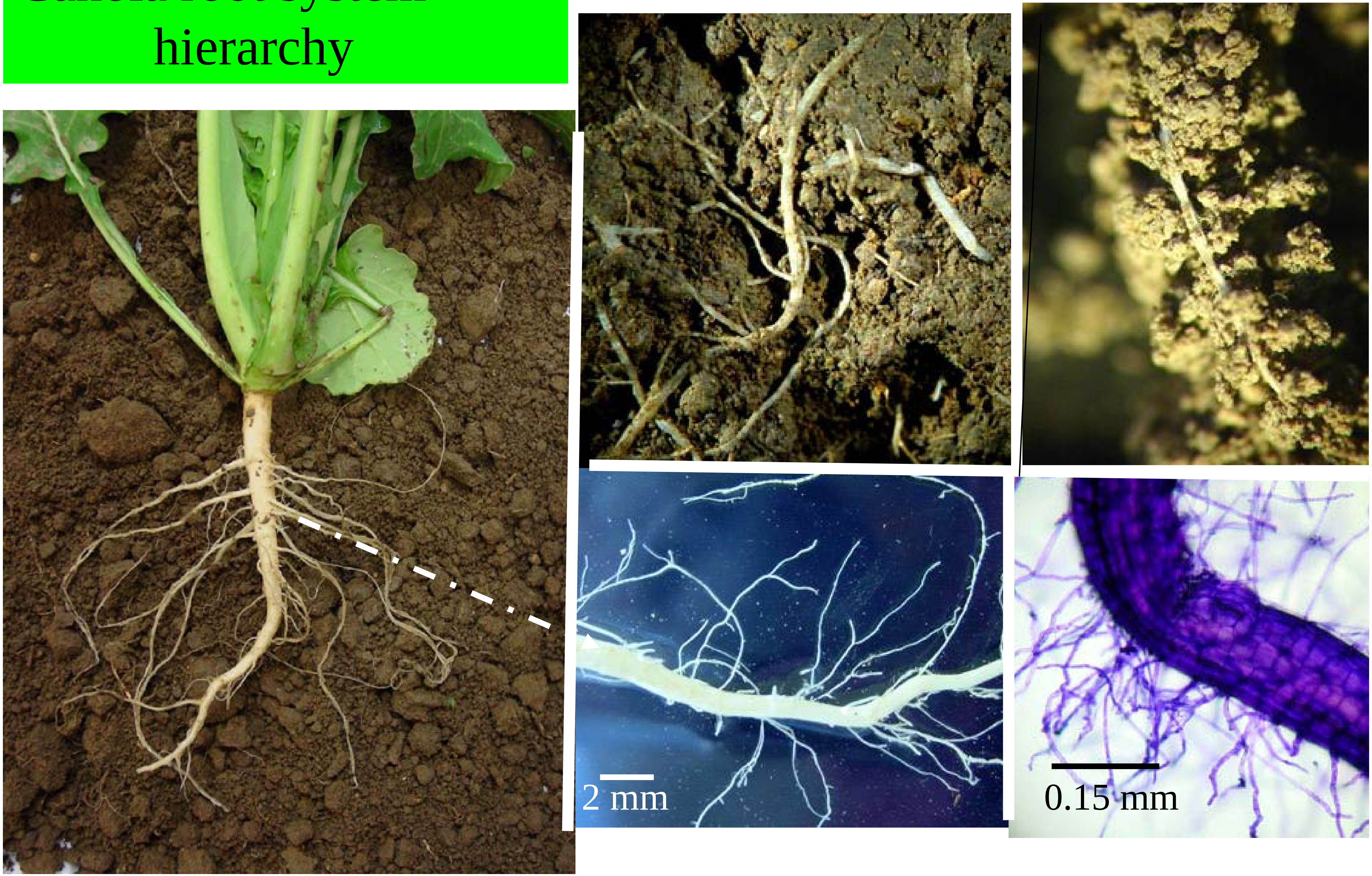
2-PE glucosinolate predominates in roots. Little or none in shoots



2-PE ITC the most toxic to soil organisms and most herbicidal

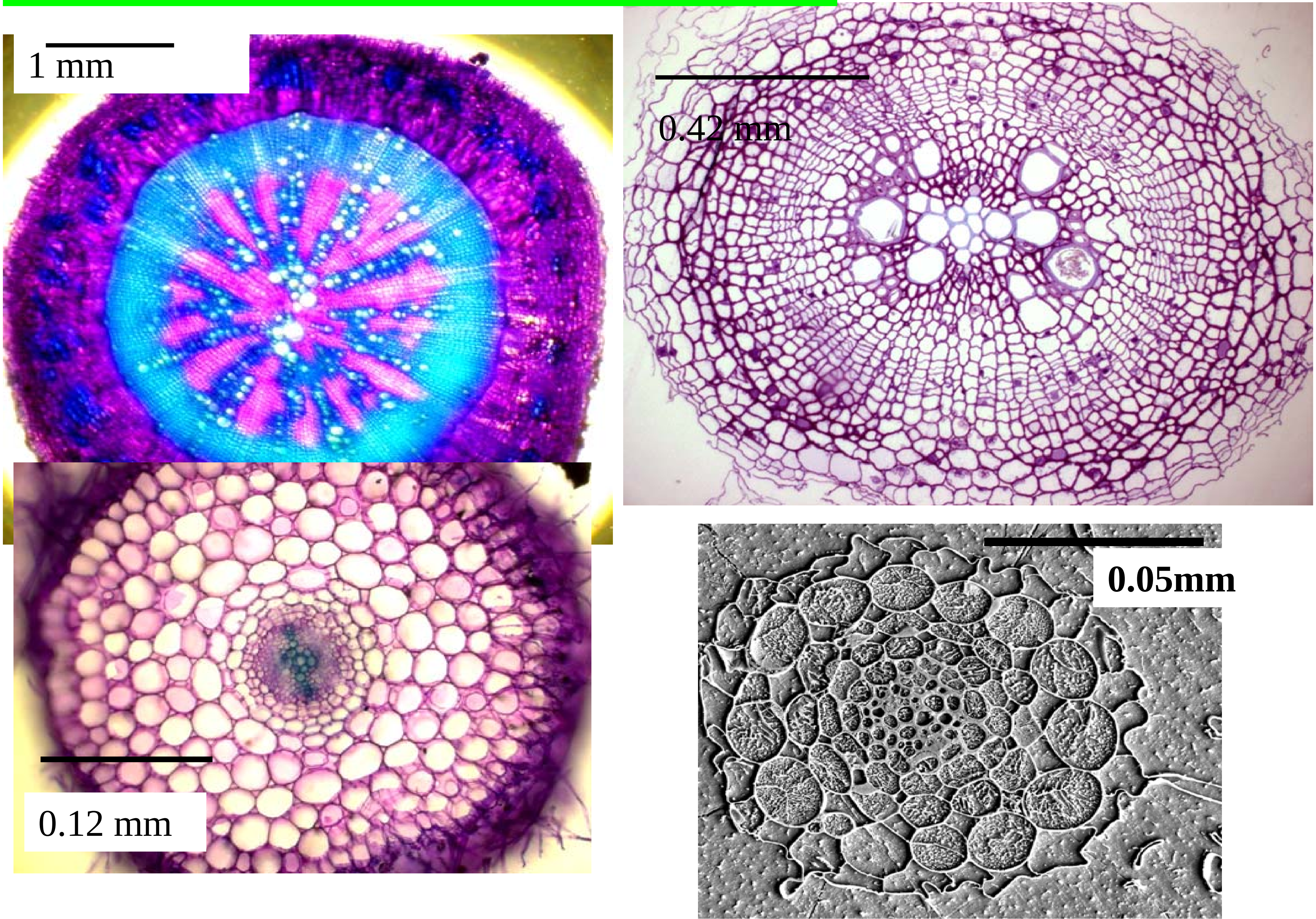
Roots are continuously exposed to potential pathogens and predators

Canola root system hierarchy



The very fine roots are short-lived and proliferate as plants mature

Component root anatomy



Localizing glucosinolates in roots

In particular root types

In different tissue regions of each root type

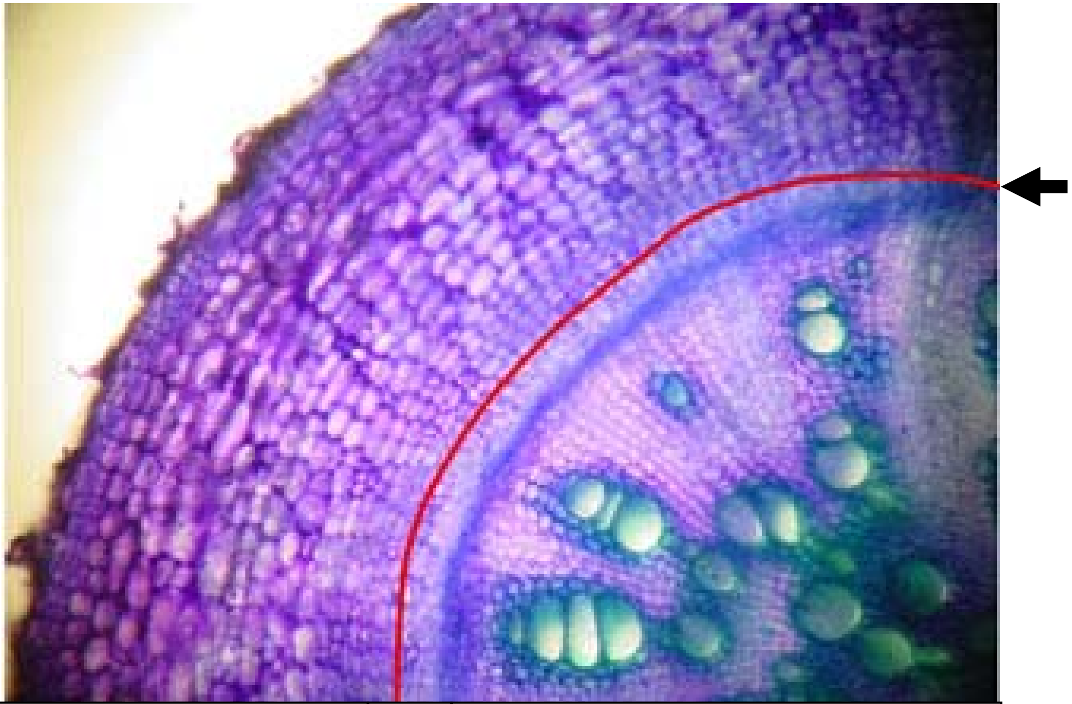
Cell-specific localization

GSL in particular root types

Varying GSL concentrations in different root types			
	Large branches >2 mm	**Tap roots**	**Small branches < 2 mm**
Concentration (µmol g_{dw}^{-1})	**15**	**7**	**7**
Contribution to root system (%)	**52%**	**35%**	**13%**

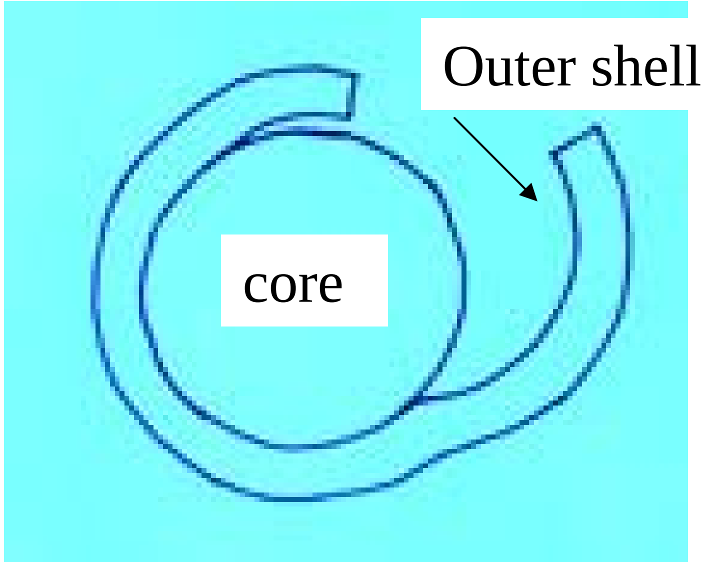
Kirkegaard & Sarwar 1999

GSL localization in two tissue regions



Periderm
Pro. Pericycle
2nd Phloem

2nd Xylem

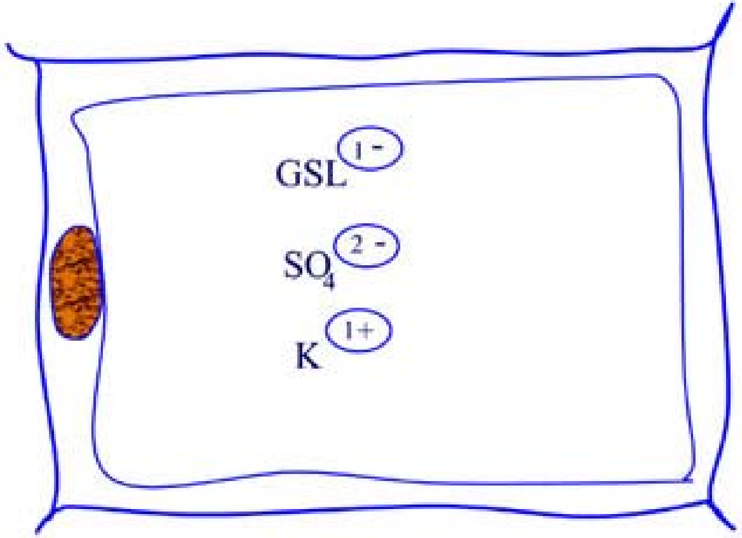


Freeze-dried roots with secondary thickening split easily in the cambial region into an inner core and outer shell

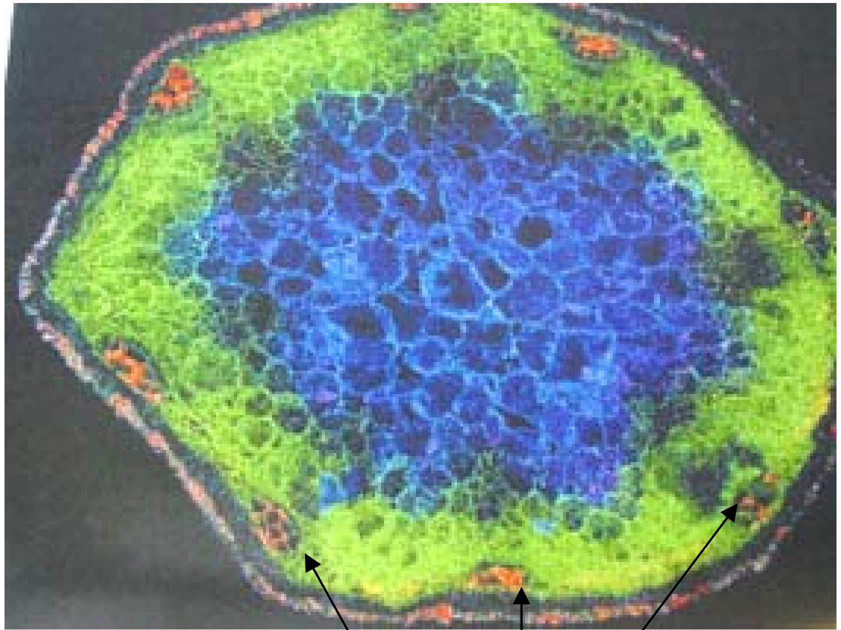
Ground pieces analysed for 2-PE GSL by HPLC

2-PE-glucosinolate content of canola root tissue regions				
Root type	**Tissue region**	**Percent total root system dw n =20**	**Glucosinolate concentration**	
			µmol g^{-1} dw	**mM**
Hypocotyl/ transition region	**Outer**	**14.2**	**11**	**2**
	Core	**25.3**	**5.5**	**1**
Rest of tap root	**Outer**	**24.9**	**14**	**2.5**
	Core	**29.2**	**6.4**	**1**
Large branch roots	**Outer**	**2.6**	**25**	**4.4**
	Core	**1.3**	**5**	**0.9**
Small branch roots	**Entire**	**2.6**	**16**	**3**

Cell-specific localization of GSL



GSL in cell vacuoles

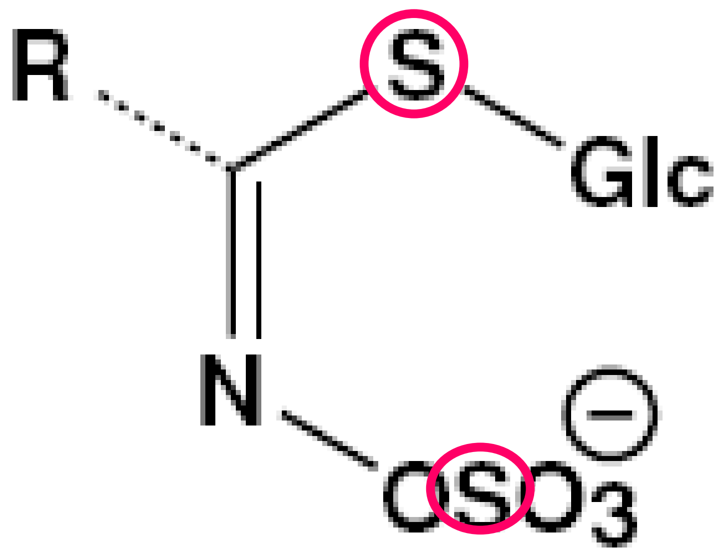


GSLs are water soluble so are only retained ***in situ*** by cryo-fixation

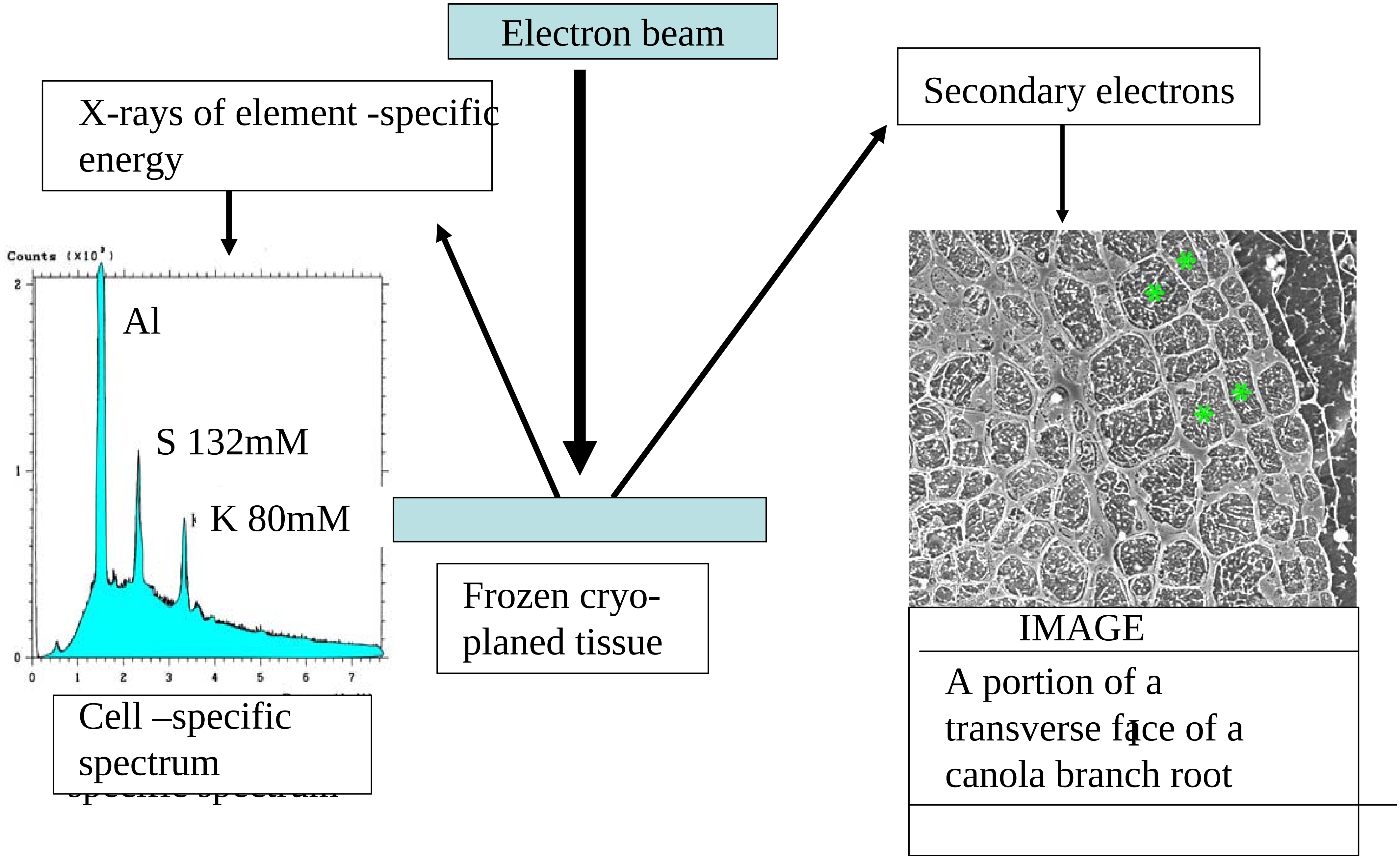
GSL localized in S cells in Arabidopsis flower stalks

Koroleva *et al* (2000)

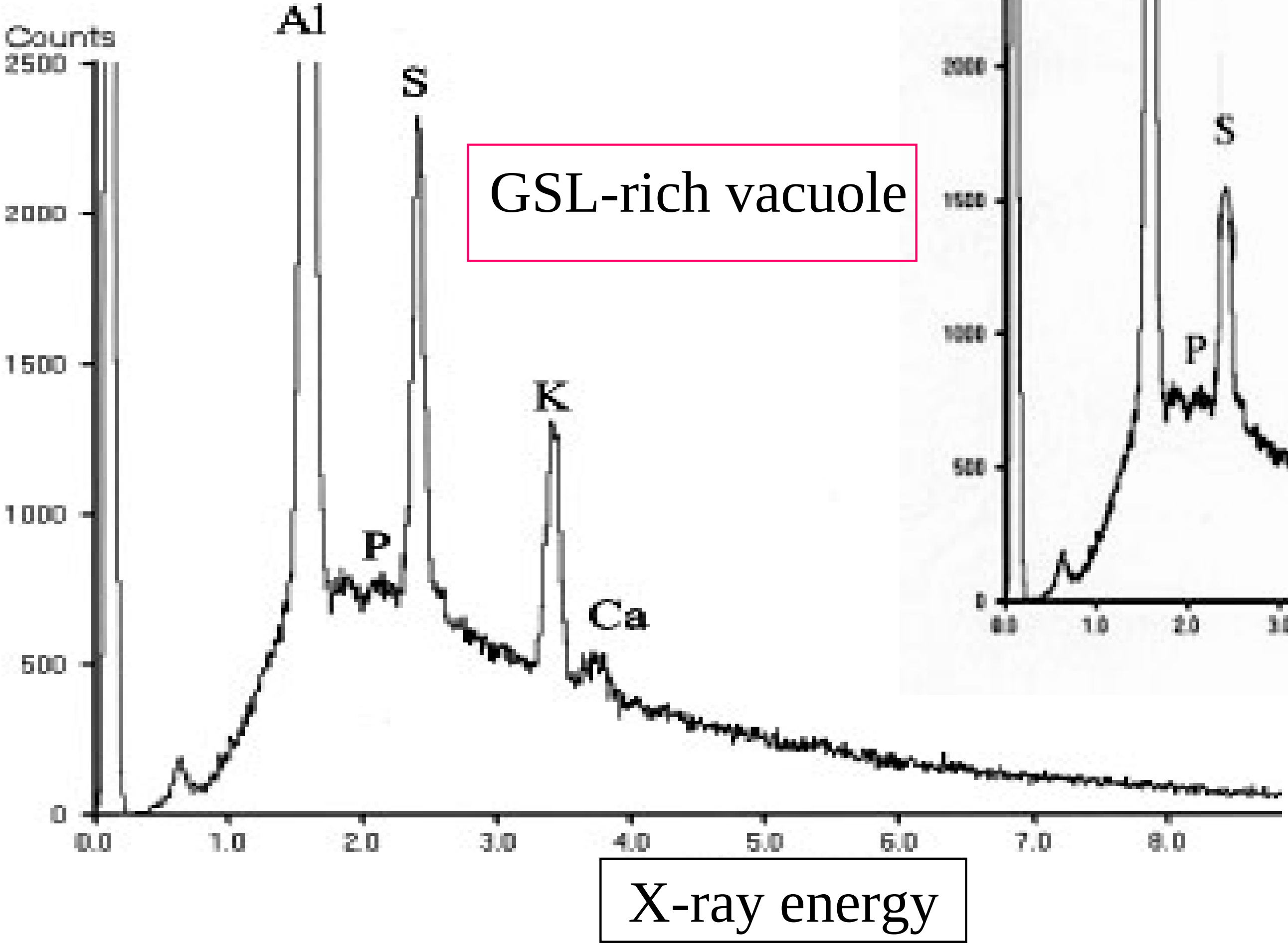
Glucosinolate structure

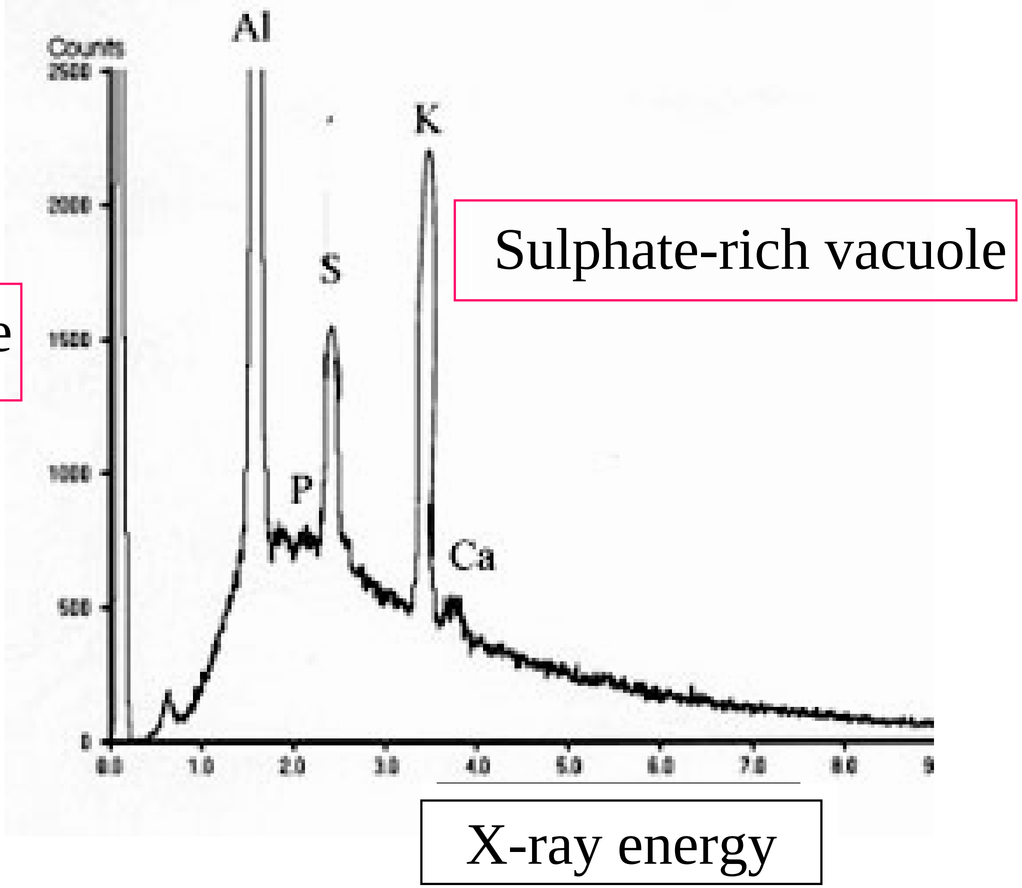


Cryo-Analytical SEM

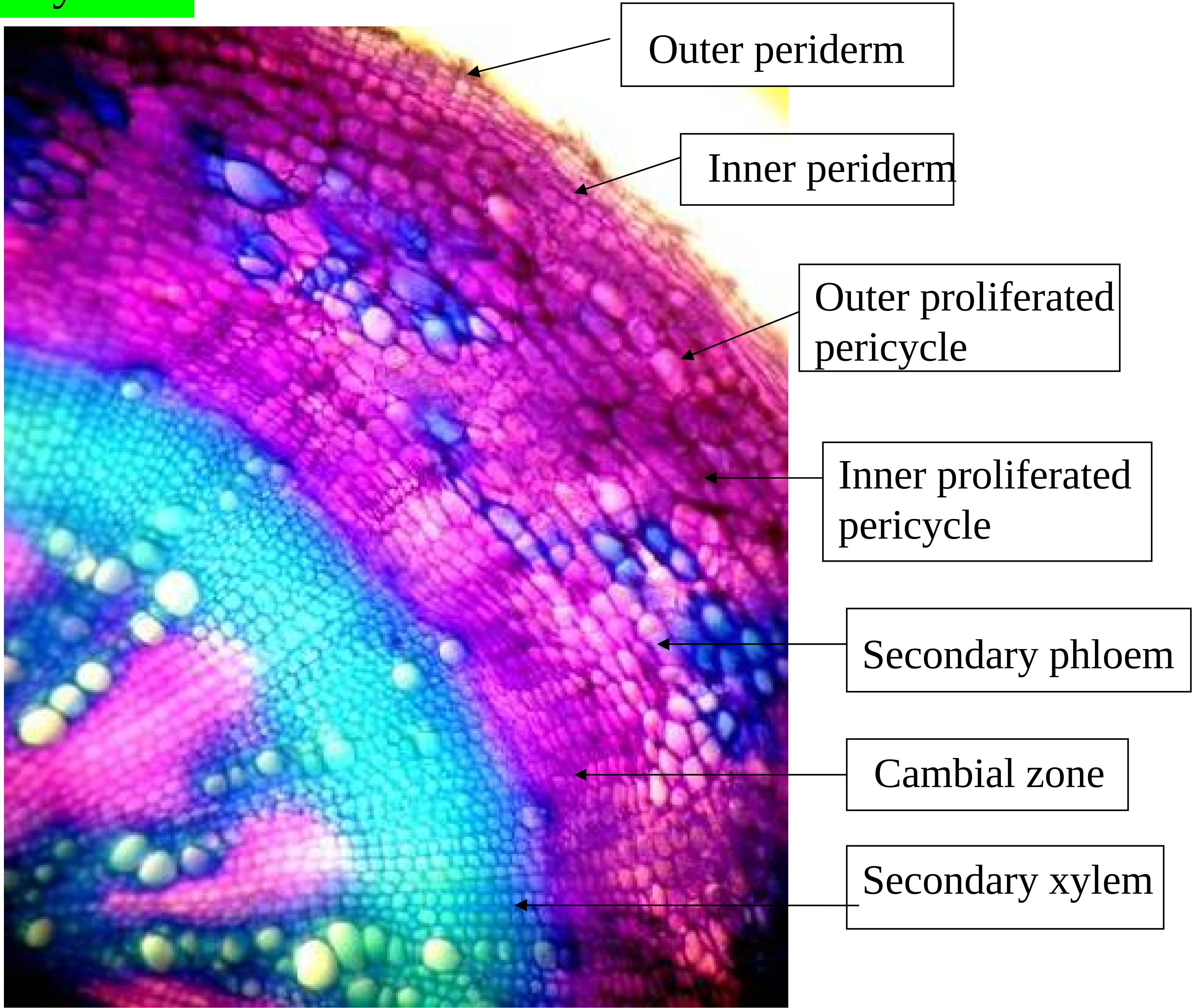


Vacuoles in GSL-rich cells have high S/K ratios





Tissues analysed



Using [S] to determine GSL concentration

$$[\,GSL\ mM\,] = \frac{[\,S\ mM\,] - 50}{2}$$

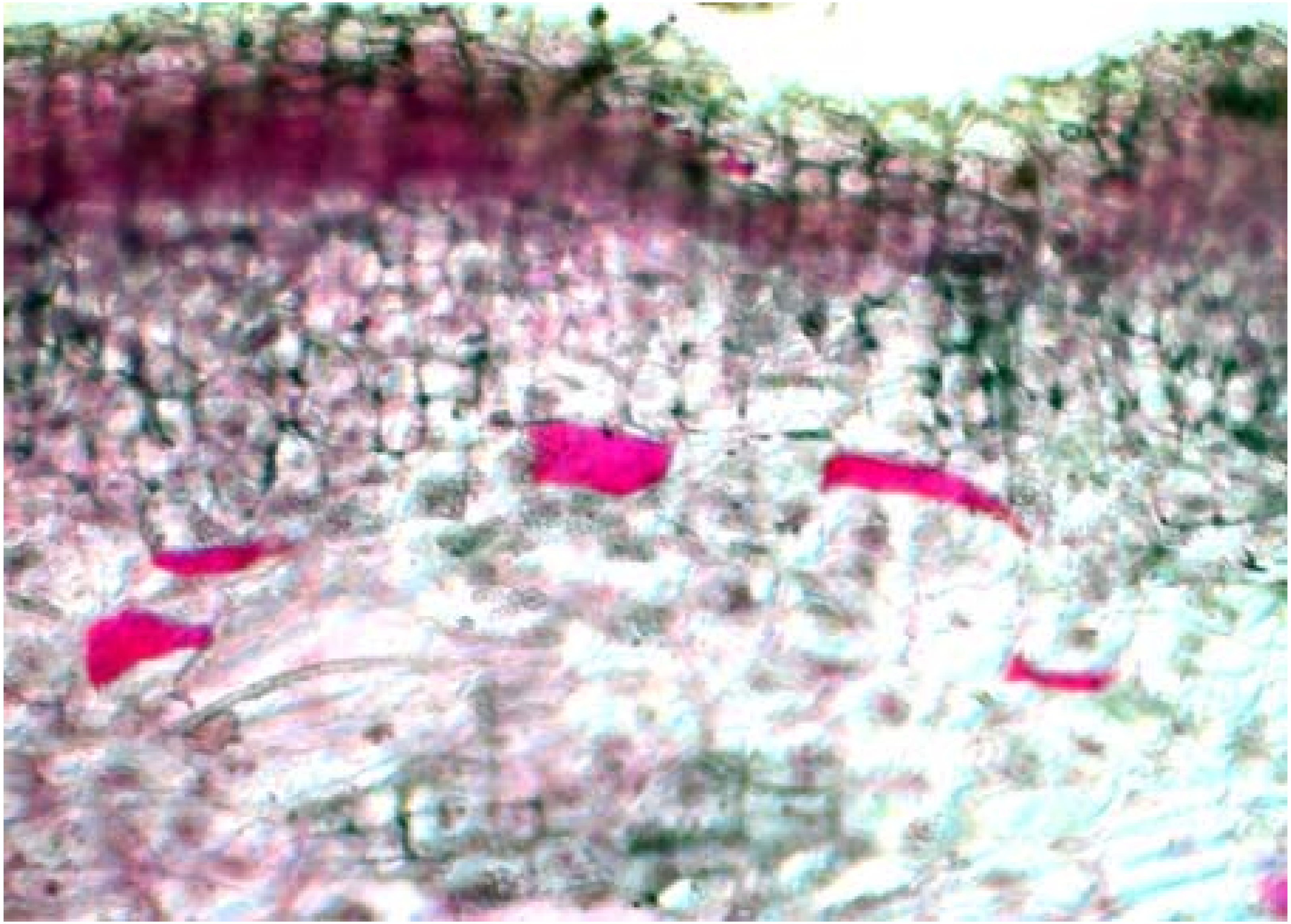
GSL a monovalent anion balanced by $1K^+$

Sulphate a divalent anion balanced by $2K^+$

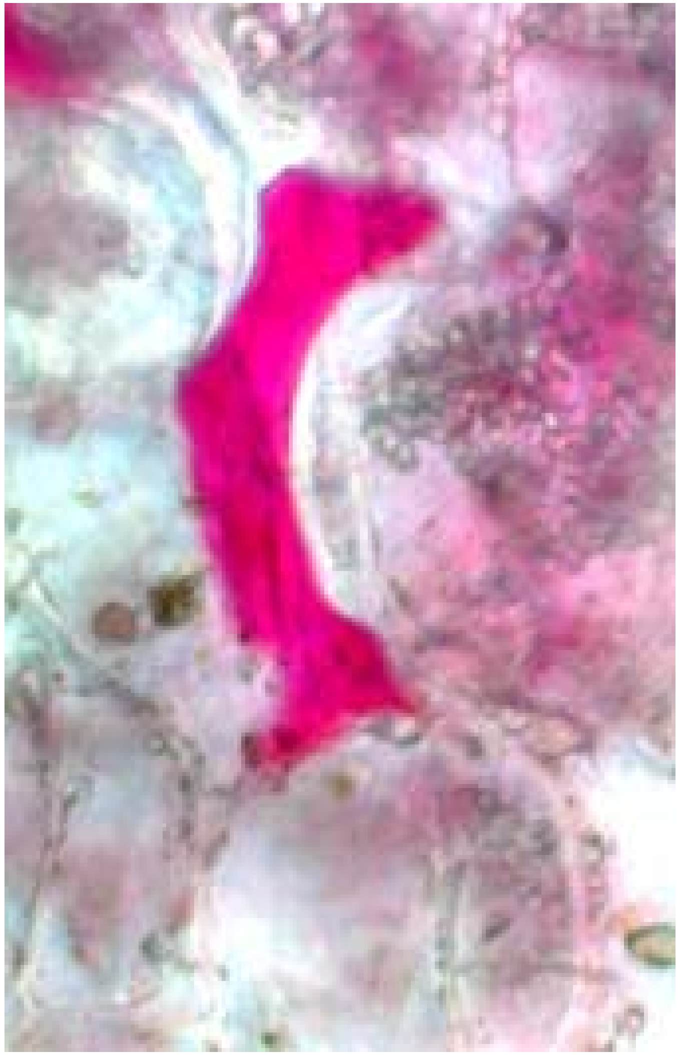
Glucosinolate concentrations in Secondary Tissues					
% of cells analysed					
Tissues	>25 mM	>50 mM	>75 mM	>100 mM	N (roots)
Outer periderm	20	7	1	0	67 (20)
Inner periderm	66	46	22	9	94 (20)
Outer pericycle	15	3	0	0	67 (20)
Inner pericycle	2	1	0	0	166 (21)
Remaining 2 ry tissues	0	0	0	0	156 (24)

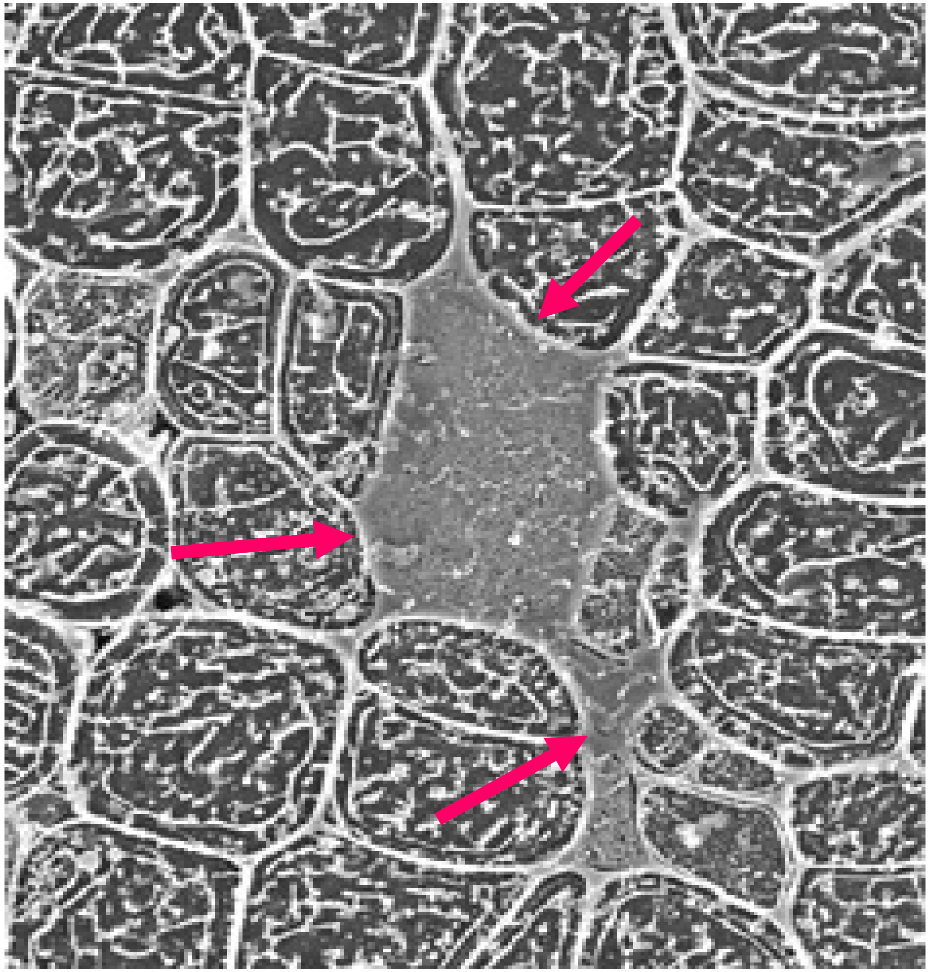
McCully et al. 2008

Myrosin cells identified by their irregular shape and strong staining for protein



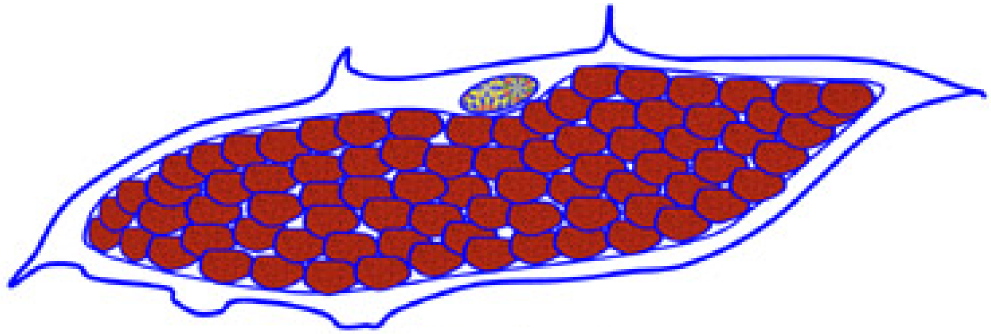
Myrosin cells easily located in the cryo-SEM





Myrosin cells

Are there GSLs in myrosin cells?



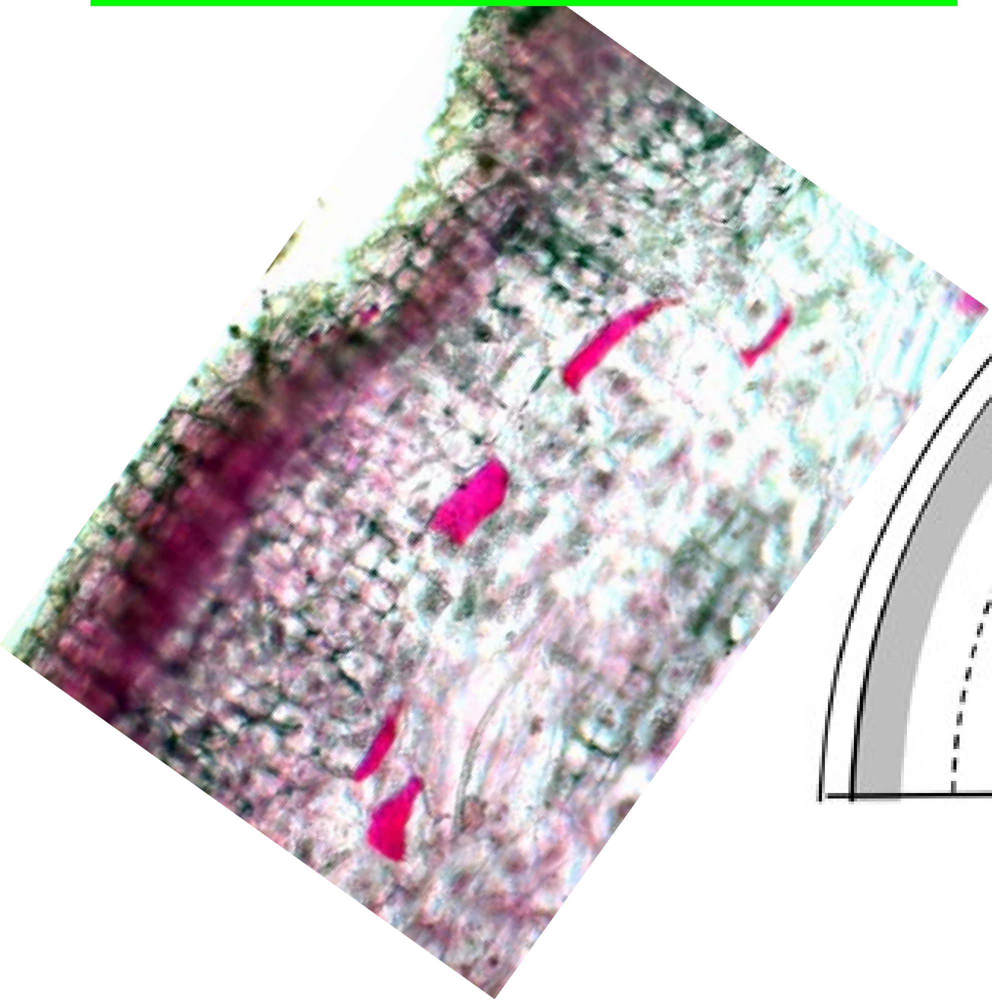
Myrosin cell

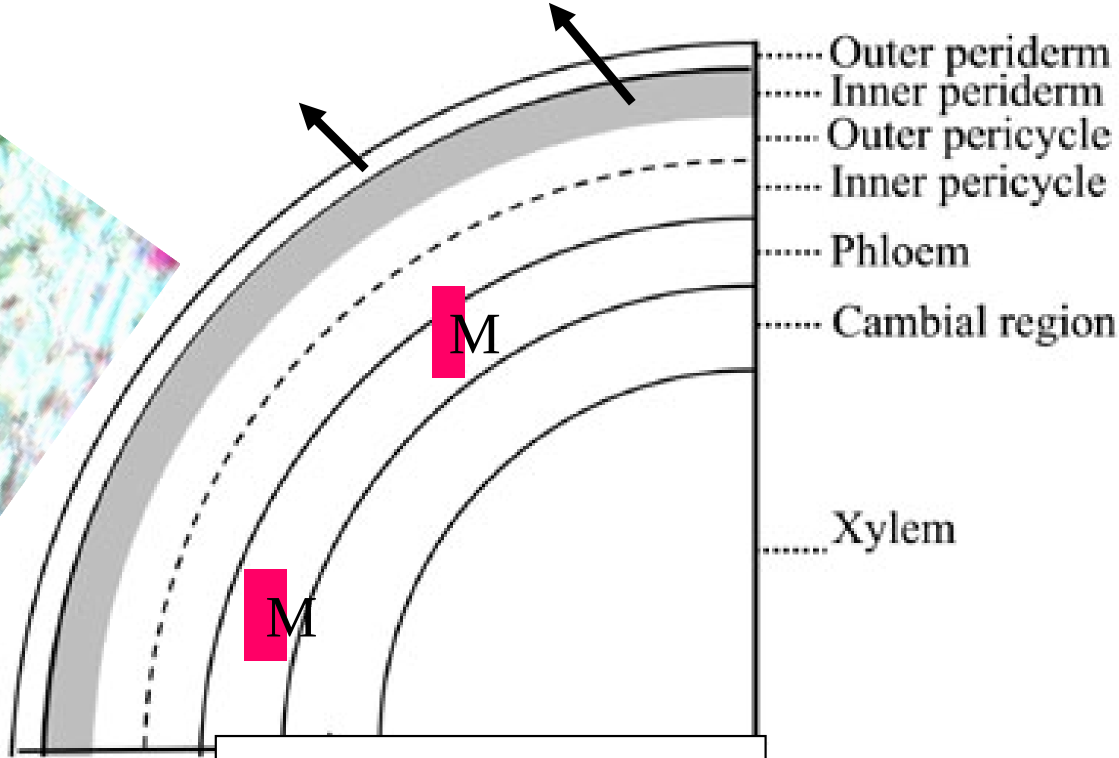
	# cells analysed	Mean [S]	Mean [K]
Myrosin cells	8	20 mM	72mM
Adjoining cells	20	32 mM	72 mM

S/K ratio for both cell types = 0.2

How does the “mustard bomb”work in undamaged growing roots?

GSL-rich periderm cells are shed and replaced as root increases in diameter





Myrosin cells remain in situ ?

Isothiocyanates in the rhizosphere ?

2 PE ITC in canola rhizospheres

Highest concentrations at bolting or flowering

Concentrations variable : 0 – 12 nmol g_{dw}^{-1}

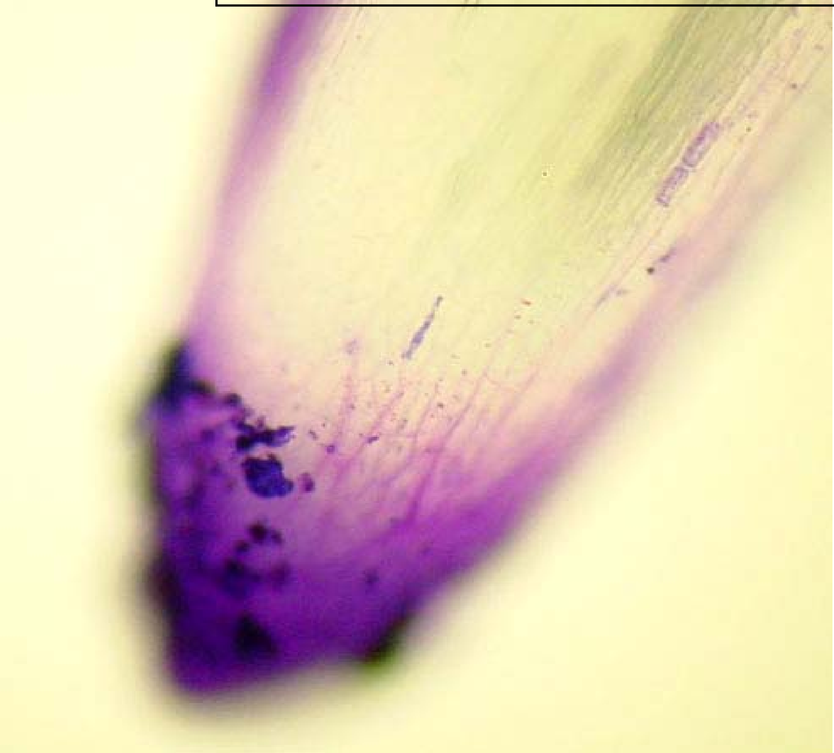
Concentrations highest next to large branch roots

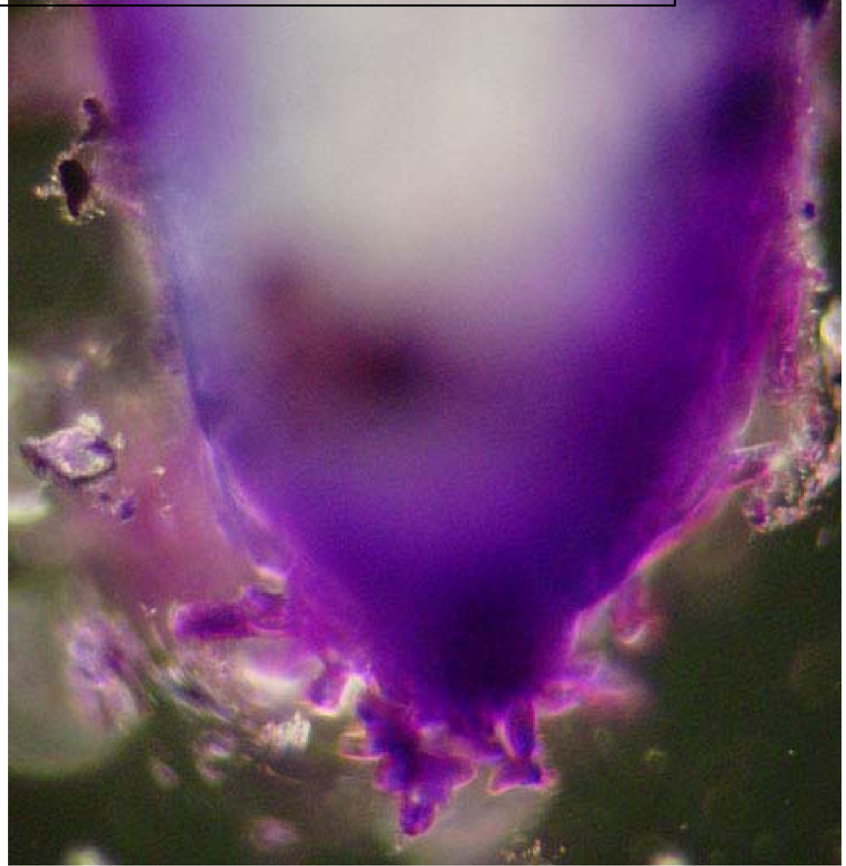
Rumberger & Marschner (2003, 2004)

Does myrosinase persist in the rhizosphere?

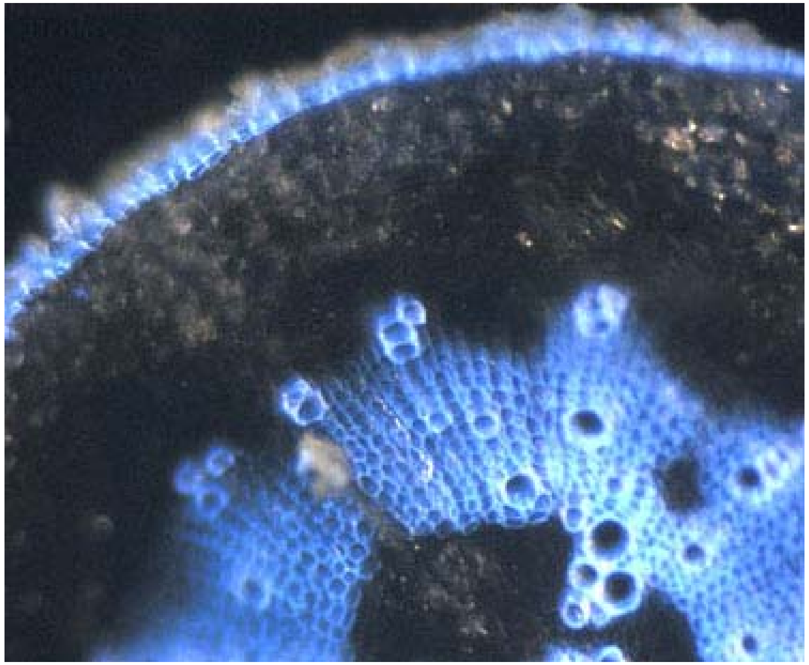
Myrosinase in the rhizosphere has been reported e.g.Borek *et al*. (1996): Al-Turki & Dick(2003)

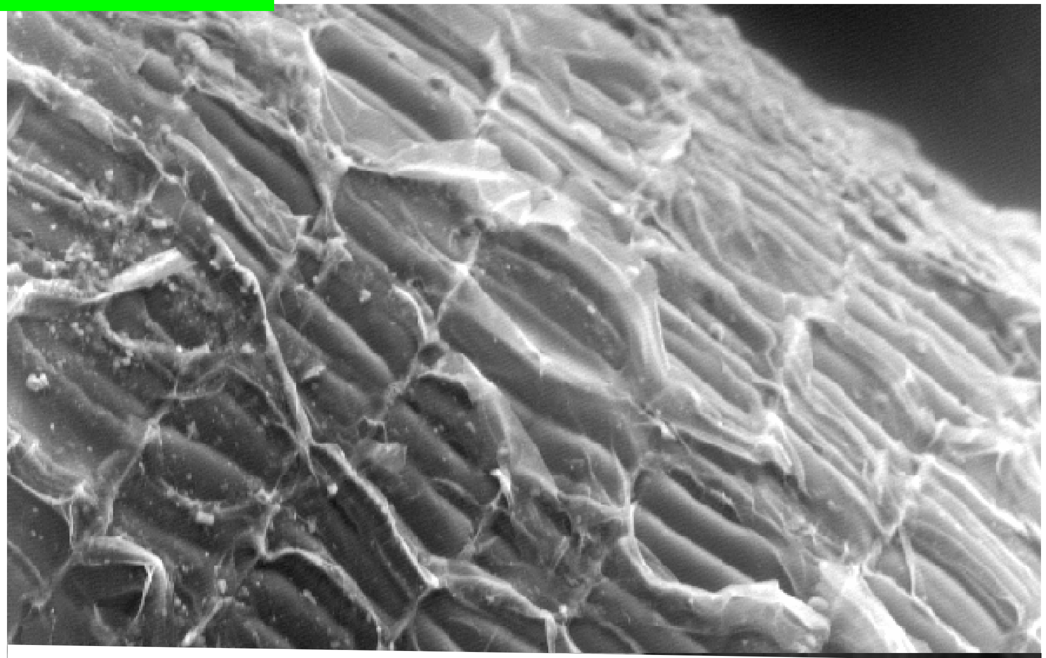
Mucilage release to the rhizosphere by canola root tips

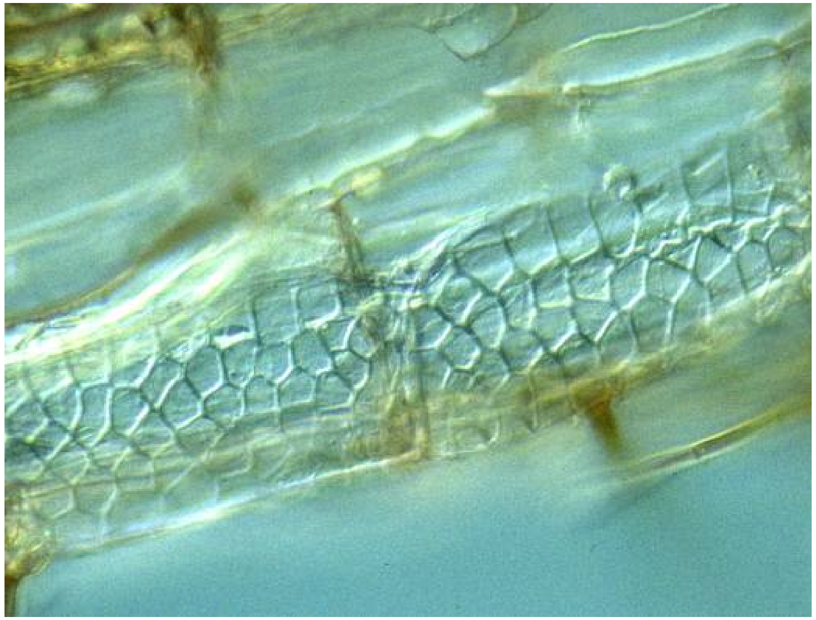




Do aliphatic-rich wall residues from periderm cells adsorb 2-PE ITC?



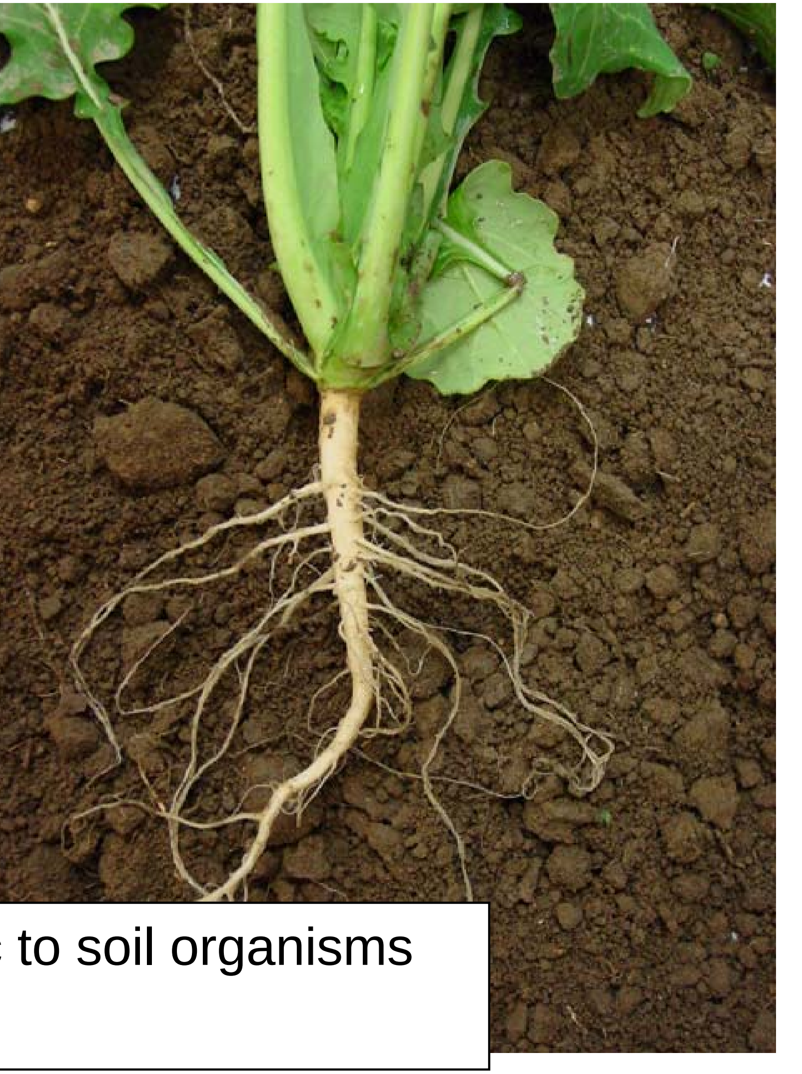




Self protection; Local biofumigation

On average roots have 4.5 X GSL concentrations in shoots

Van Dam *et al*. 2008

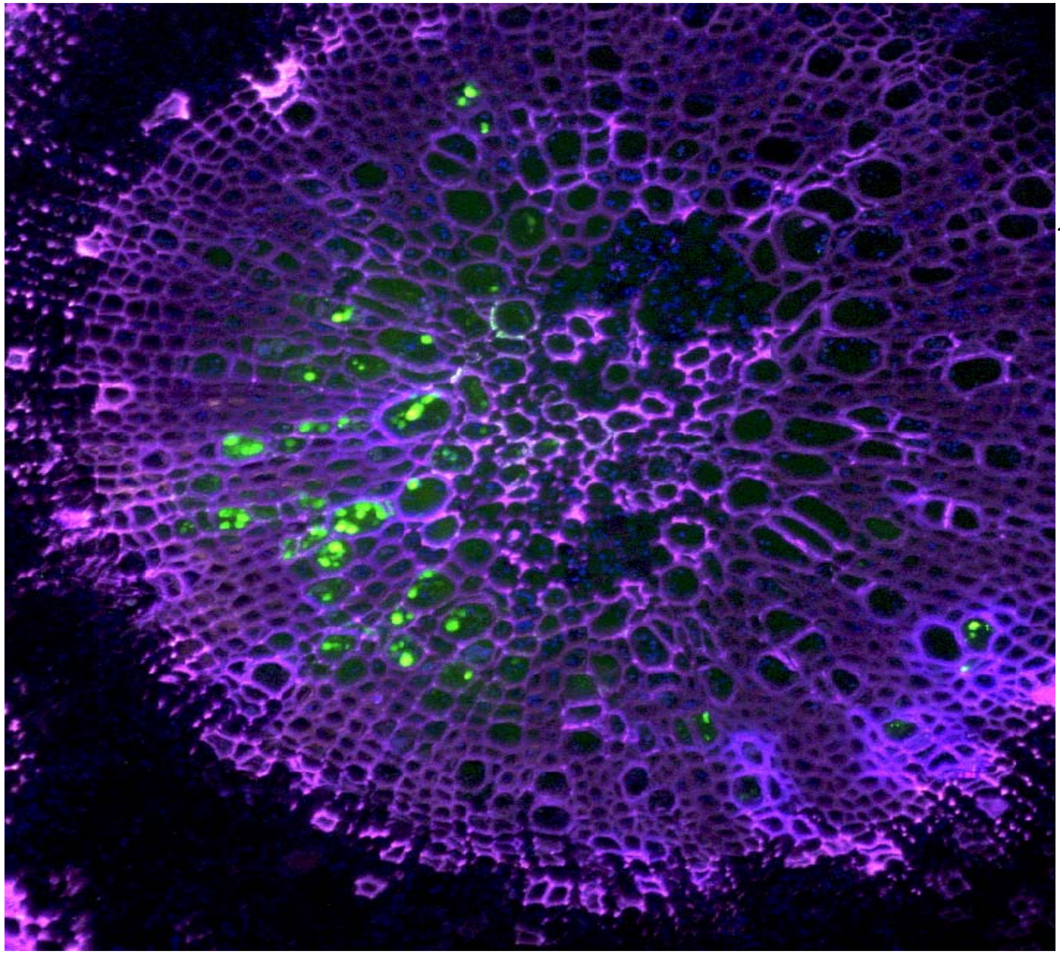


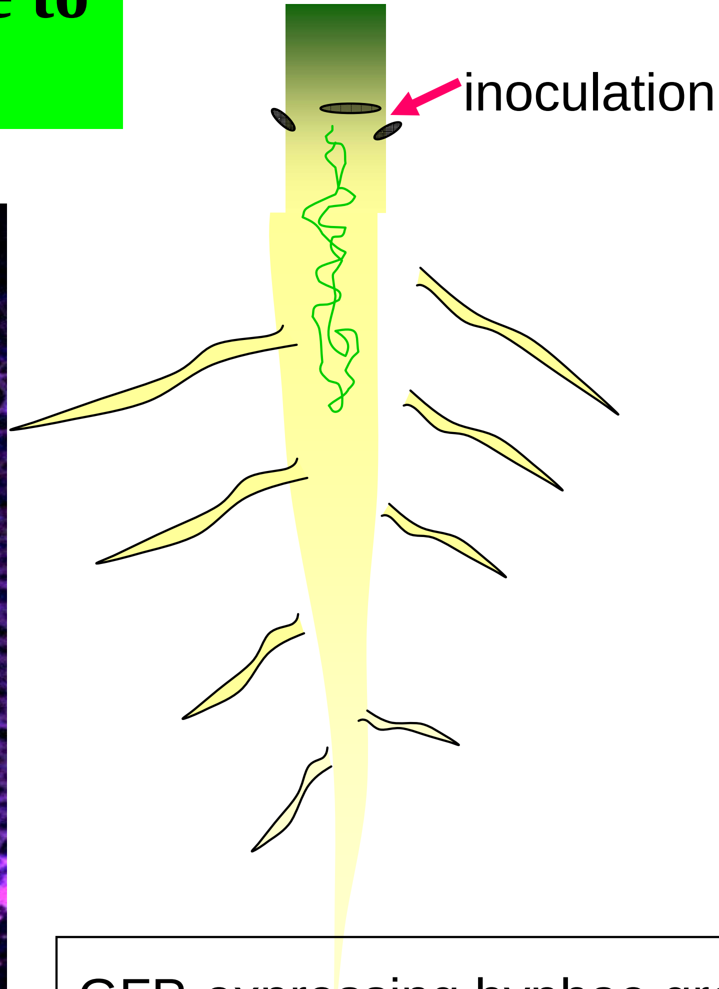
2-PE glucosinolate predominates in roots. Little or none in shoots

2-PE ITC the most toxic to soil organisms and most herbicidal

Roots are continuously exposed to potential pathogens and predators

Does peripheral GSL positioning help confine *Leptosphaeria maculens* hyphae to main root core till post-flowering?





GFP-expressing hyphae grow downward into the root secondary xylem core

Susan Sprague 2007