Genetic Engineering of Plant Selenium Metabolism for Phytoremediation

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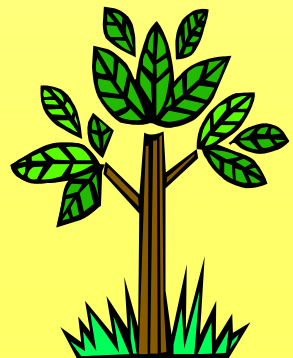
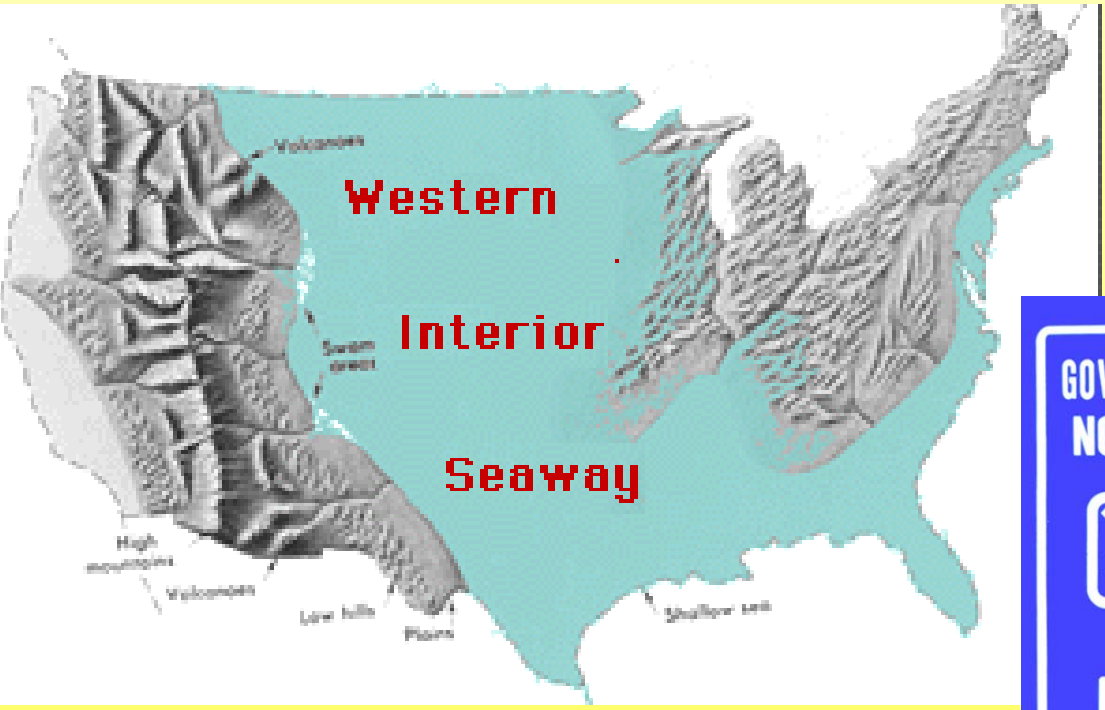
Outline

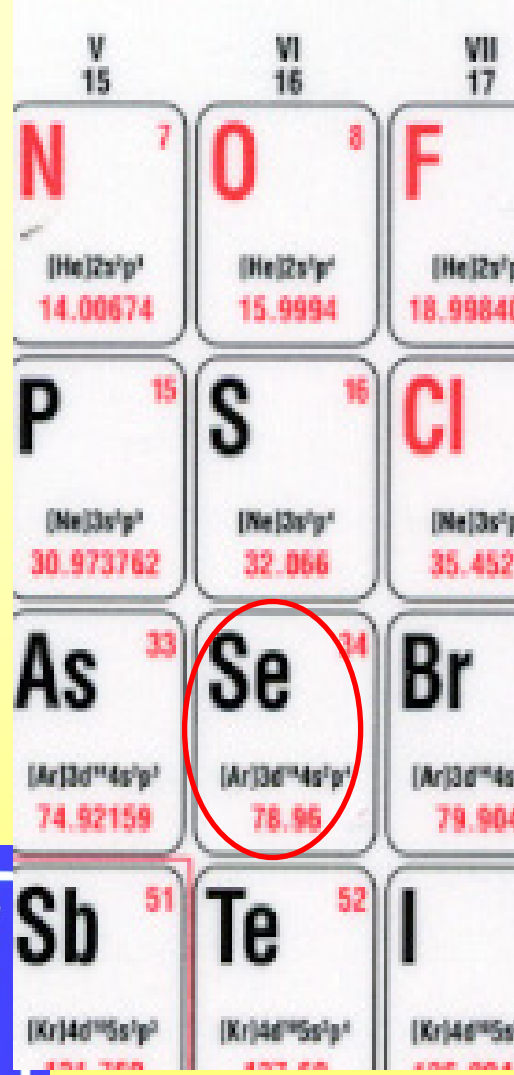
- Introduction of plant Selenium metabolism
- Genetic engineering of plant Se metabolism
- Testing transgenics for Se phytoremediation
- Ecological aspects of plant Se accumulation



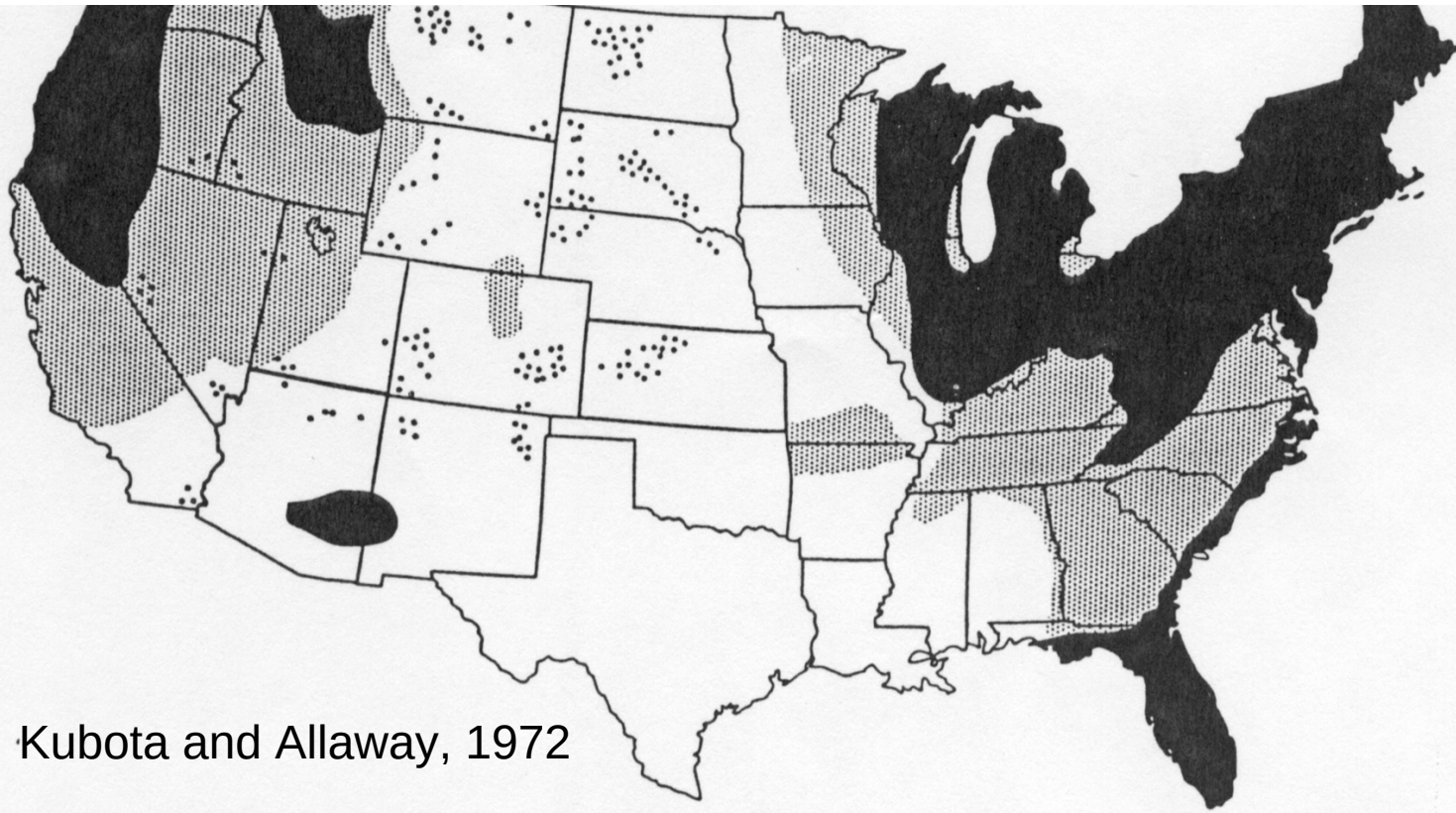
Selenium

- Nutrient
- Toxin
- Pollutant





Selenium deficiency and toxicity are both problems in the U.S.



Kubota and Allaway, 1972

Low-approximately 80% of all forage and grain contain <0.05 ppm of selenium.

Variable-approximately 50% contains >0.1 ppm.

Adequate-80% of all forages and grain contain >0.1 ppm of selenium.

- Local areas where selenium accumulator plants contain >50 ppm.

What can plants do with selenium?

selenate → selenite → Se-cysteine → Se-methionine → dimethylselenide

sulfate → sulfite → cysteine → methionine → dimethylsulfide

proteins

accumulation
volatilization

In some plants:
hyperaccumulation

Stanleya pinnata

Genetic engineering of plant Se metabolism

selenate

↓ *ATP sulfurylase(APS)*

selenite

↓

proteins ← (blocked)

Se-cysteine

← *Se-cysteine lyase (SL)*

alanine + Se^0

↓ *Cystathionine γ synthase (CγS)*

Se-methionine

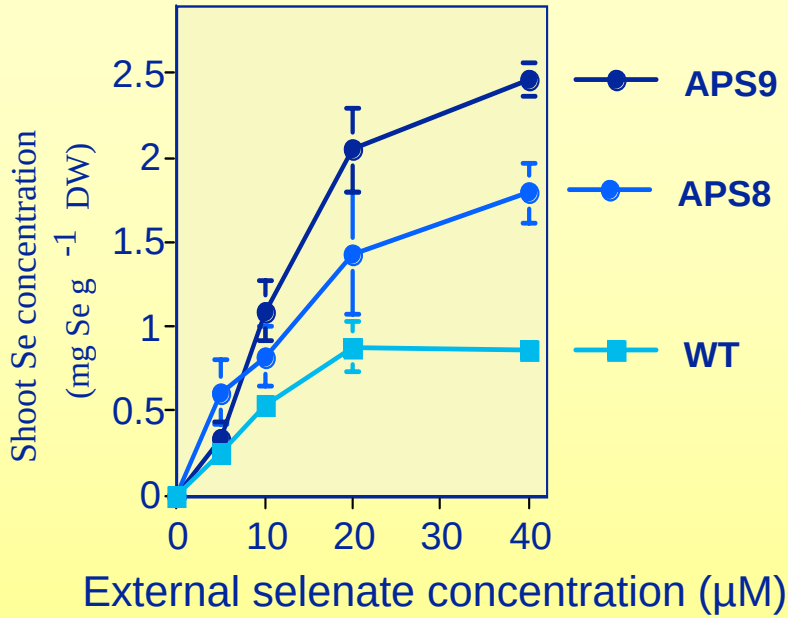
↓

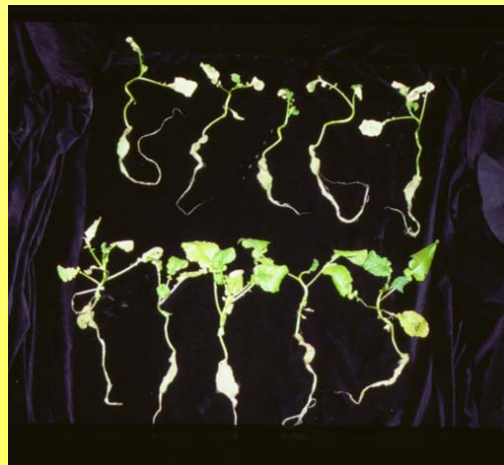
dimethylselenide ↑

Brassica juncea

APS overexpression leads to:

- enhanced Se accumulation
  - as organic Se
- enhanced Se tolerance
- no difference in Se volatilization





selenate

ATP sulfurylase(APS)

selenite

proteins

alanine + Se^0

Se-cysteine lyase (SL)

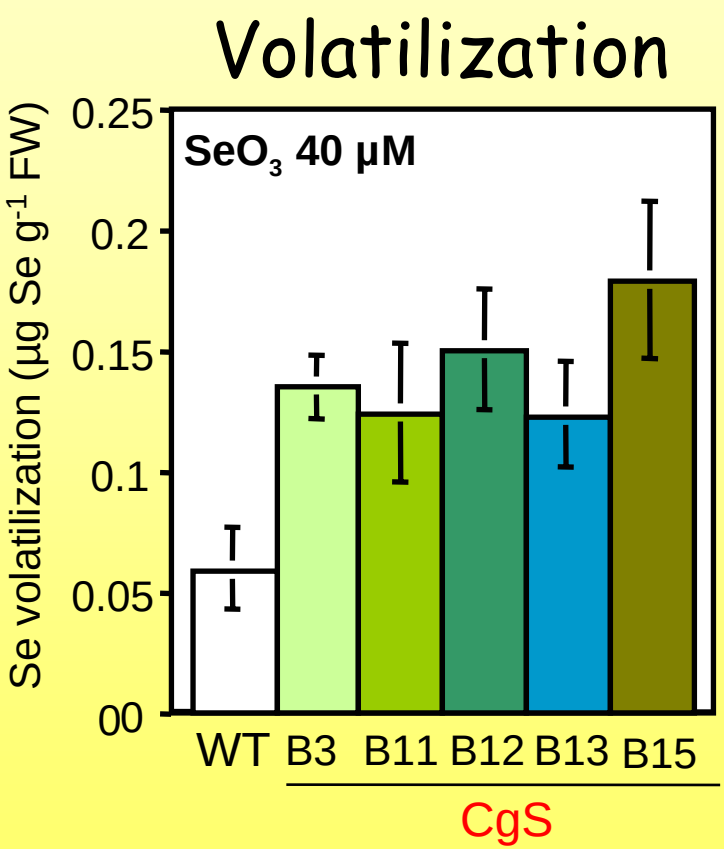
Se-cysteine

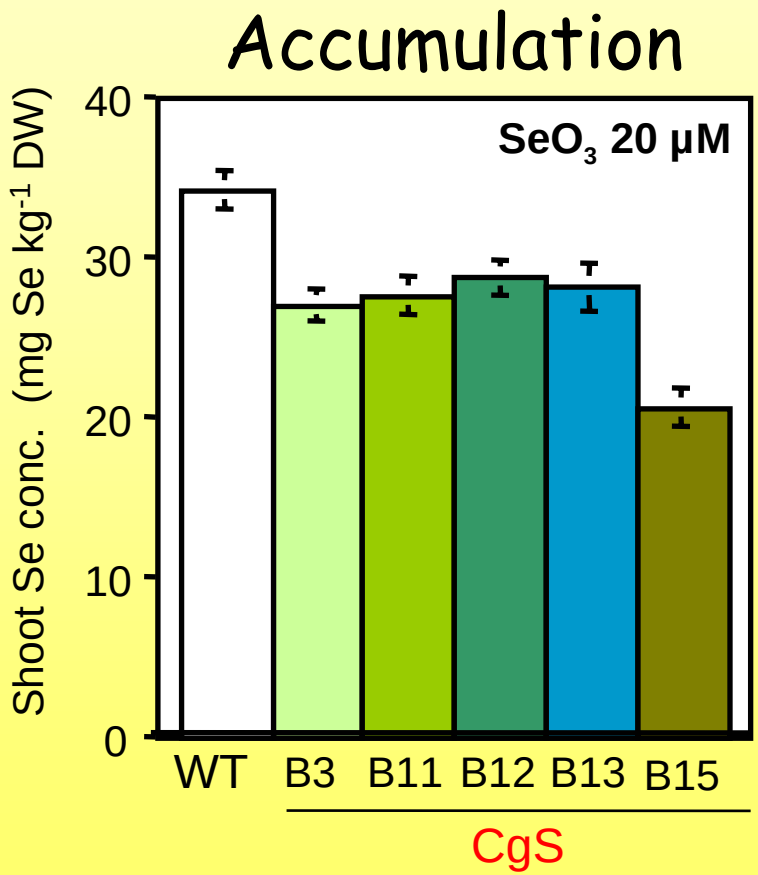
Cystathionine γ synthase (CγS)

Se-methionine

dimethylselenide

CgS transgenics: volatilize more Se, accumulate less Se





selenate

↓ *ATP sulfurylase(APS)*

selenite

proteins

↓

alanine + Se^0 ← Se-cysteine

Se-cysteine lyase (SL)

↓ *Cystathionine γ synthase (CγS)*

Se-methionine

↓

↓

dimethylselenide ↑

Jennifer Owen

Gulnara Garifullin

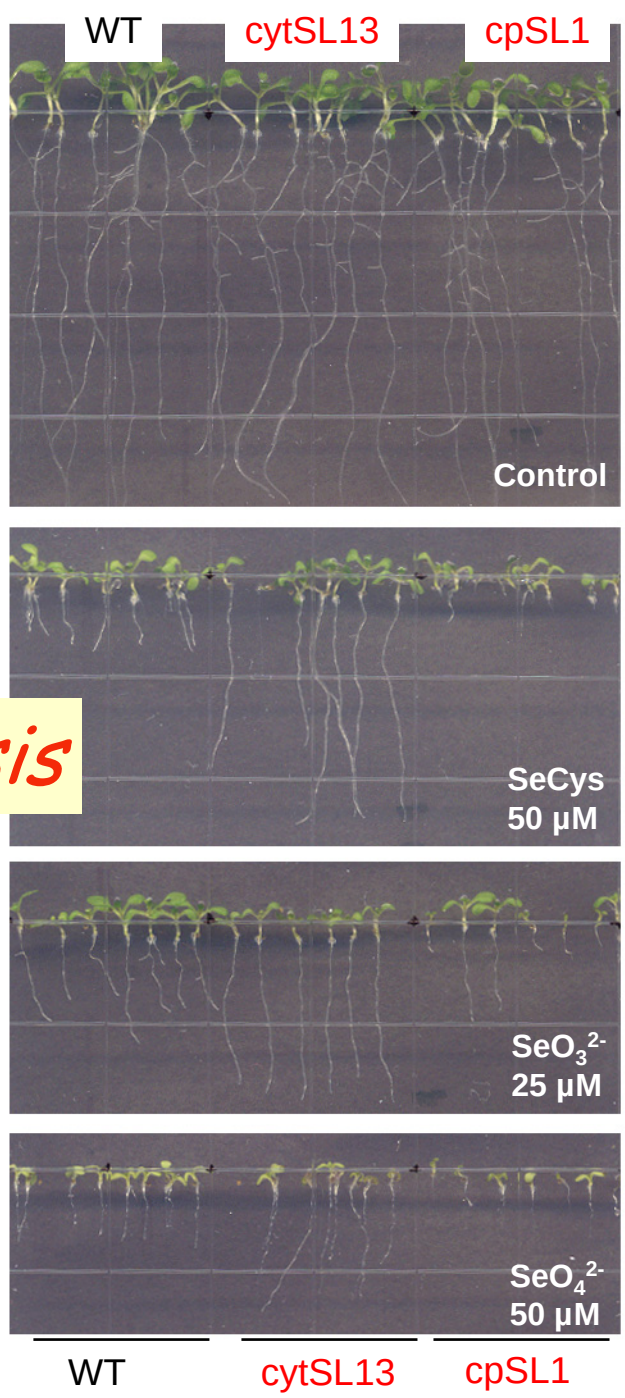
CytSL Lines:

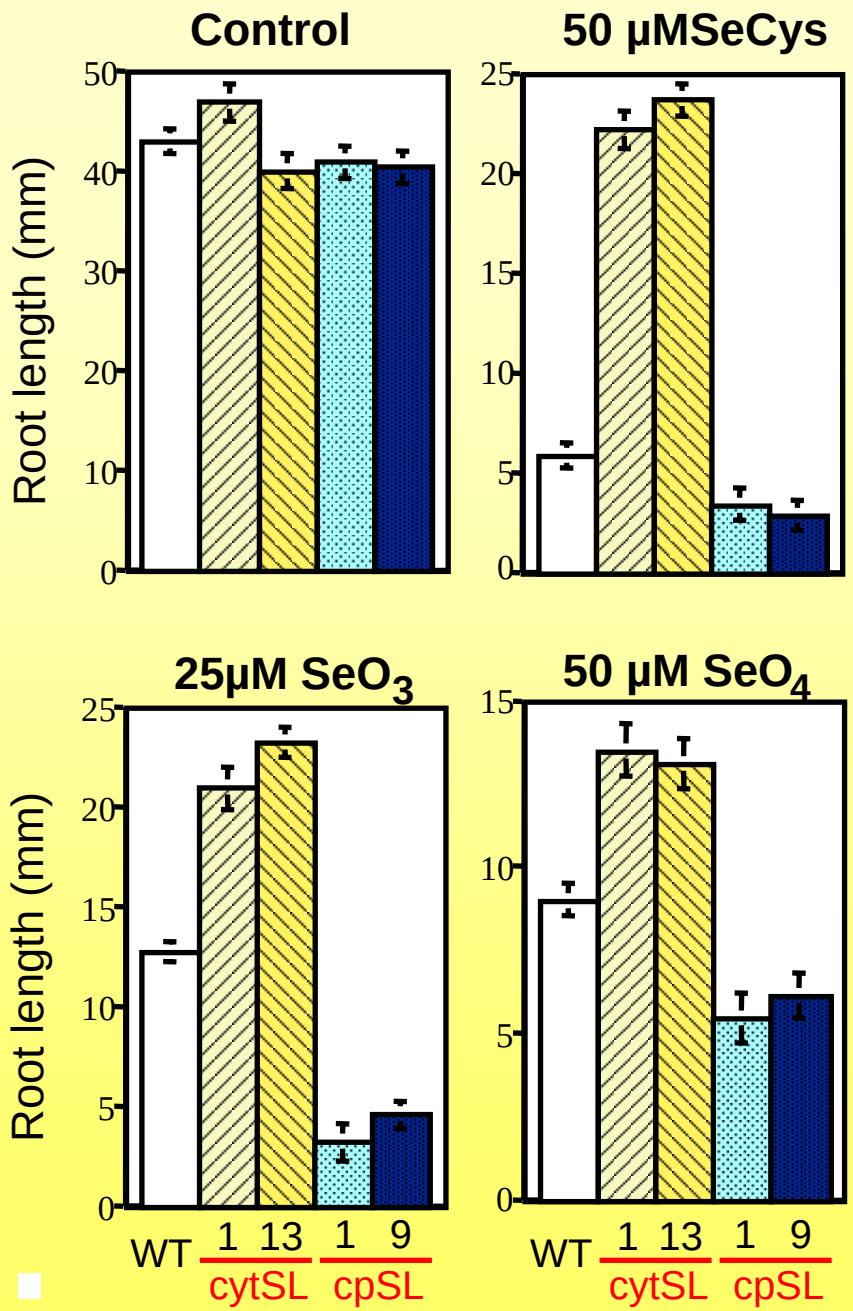
Enhanced Se tolerance

Arabidopsis

CpSL lines:

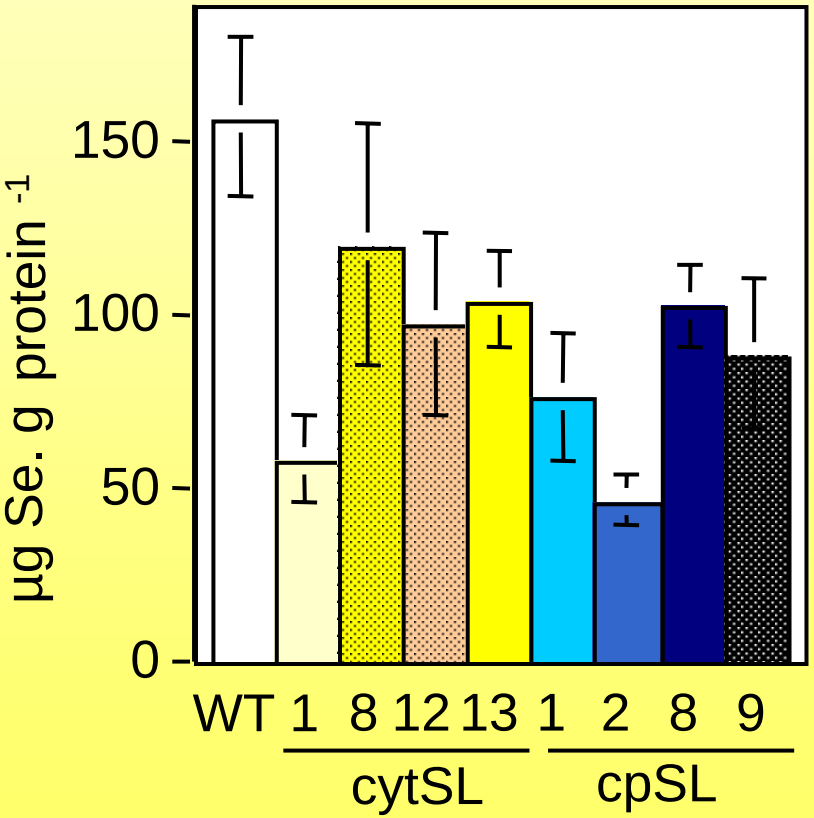
Reduced Se tolerance



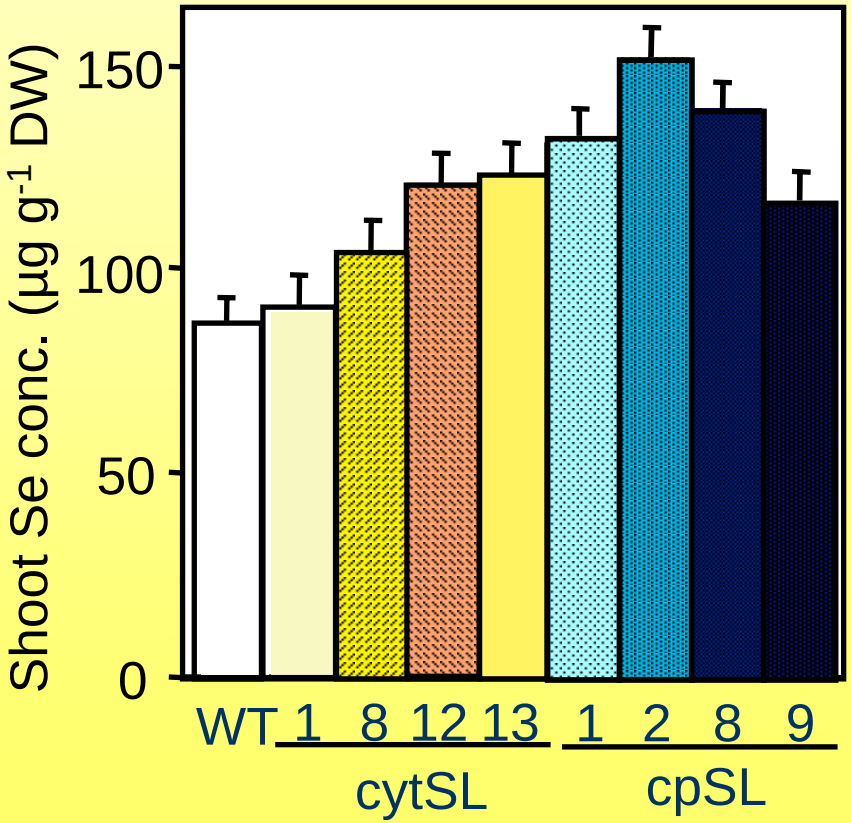


Arabidopsis cytSL & cpSL transgenics:

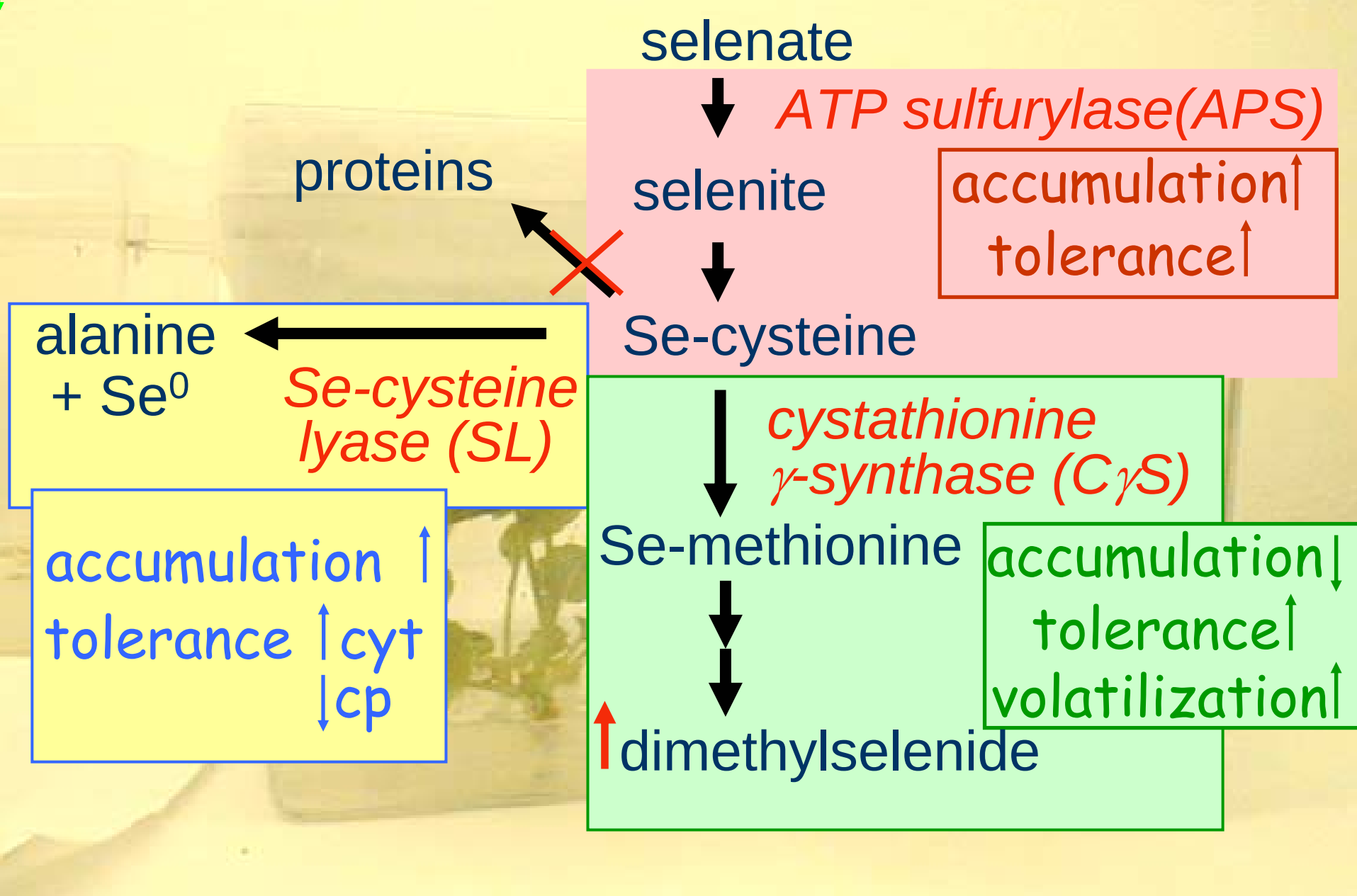
have less Se in protein



accumulate more Se

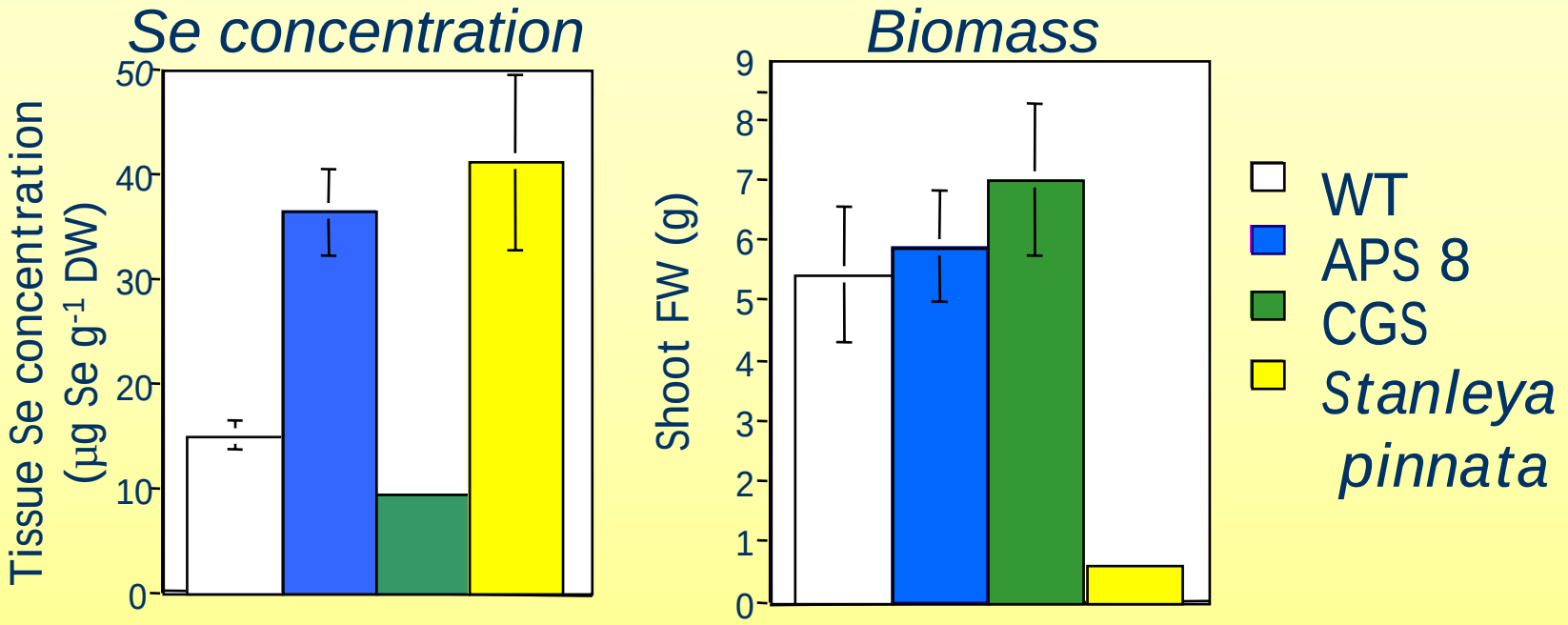


Genetic engineering of plant Se metabolism



Testing transgenics for phytoremediation

Greenhouse pot experiment using Se-rich CO soil

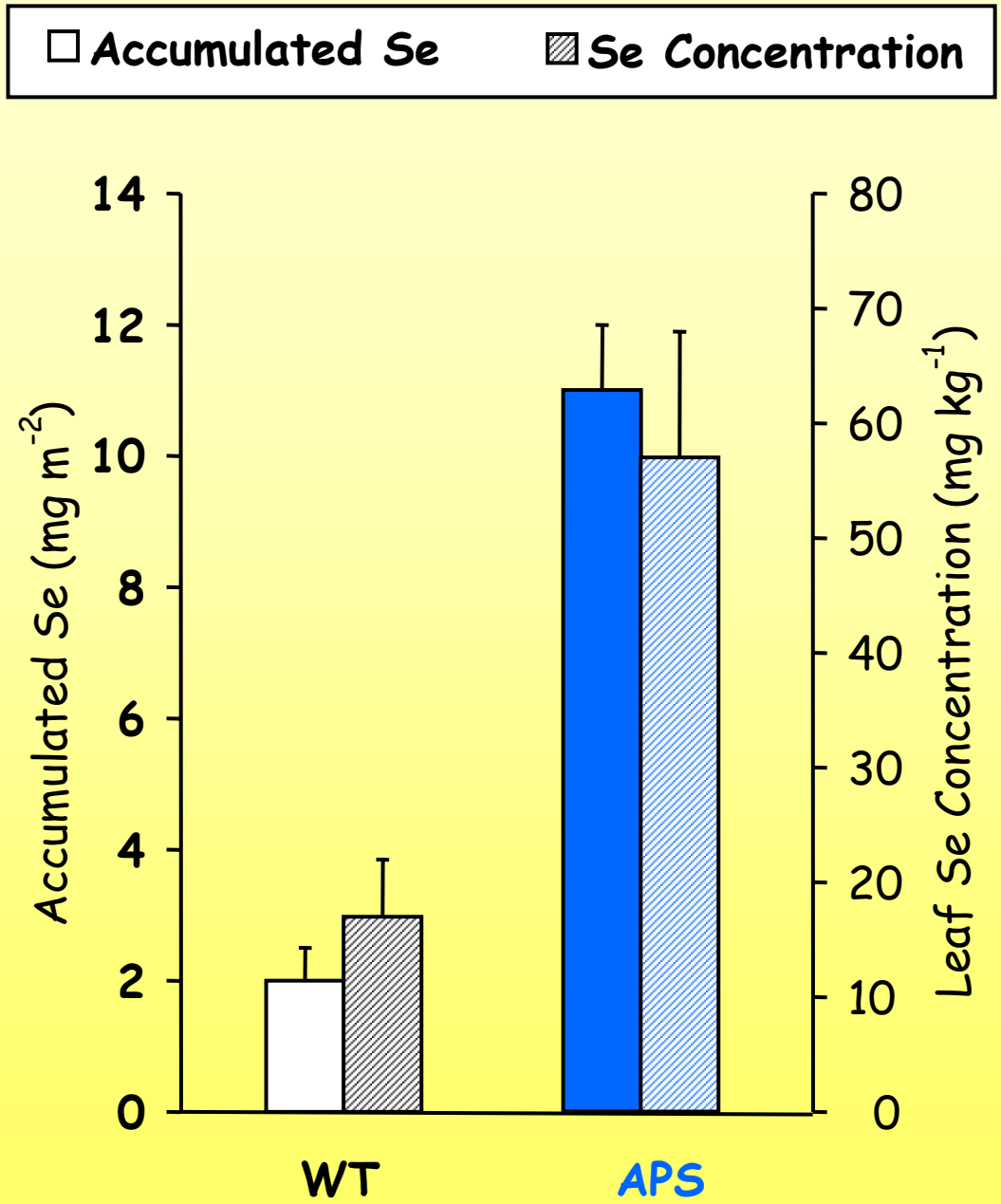


- APS transgenics accumulated 3-fold more Se than wildtype plants

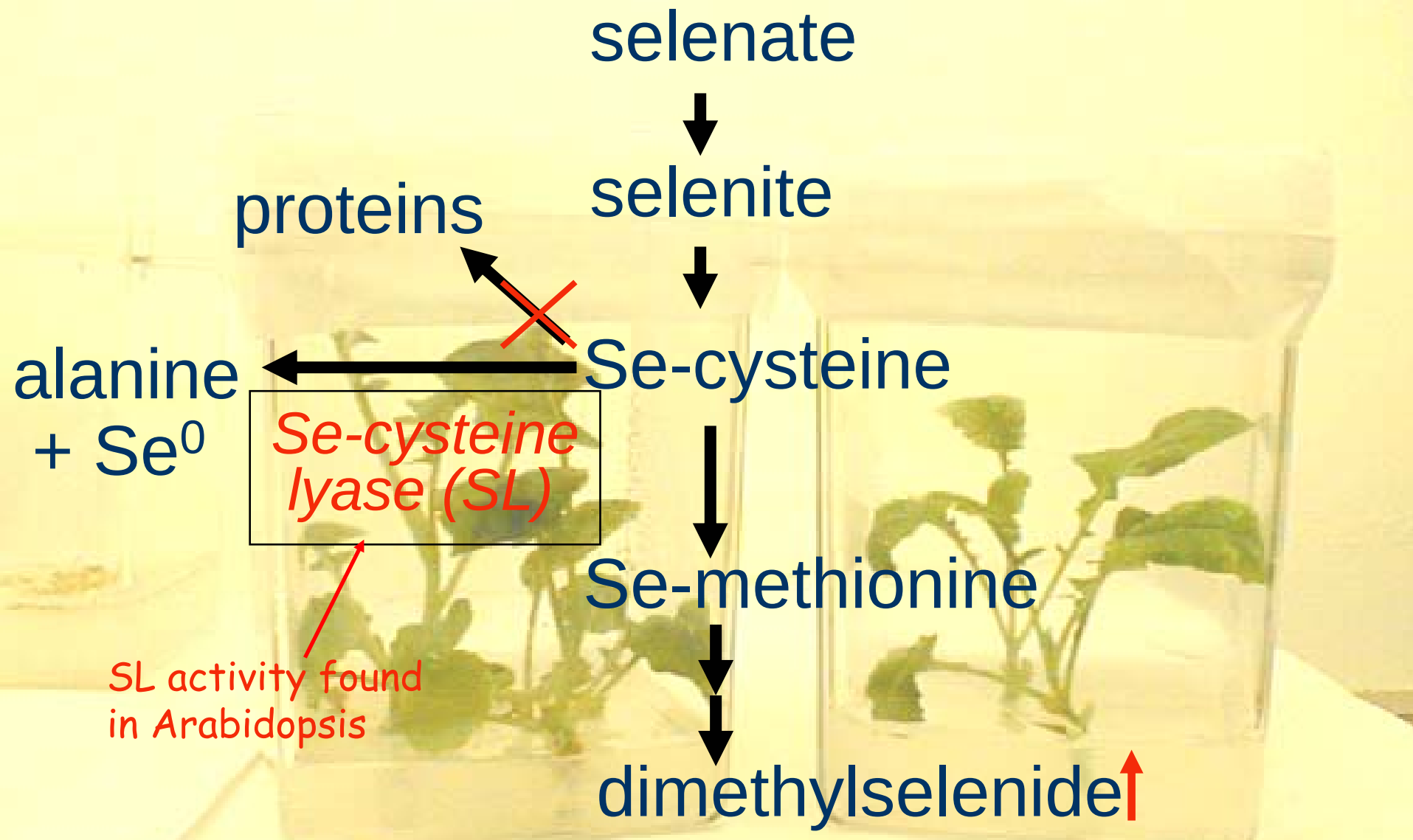
- CgS transgenics accumulated ~40% less Se than wildtype plants
  (due to higher Se volatilization?)

Field experiment *in CA central valley*

APS transgenics accumulated 5-fold more Se than wildtype plants



Identification of new genes involved in Se metabolism

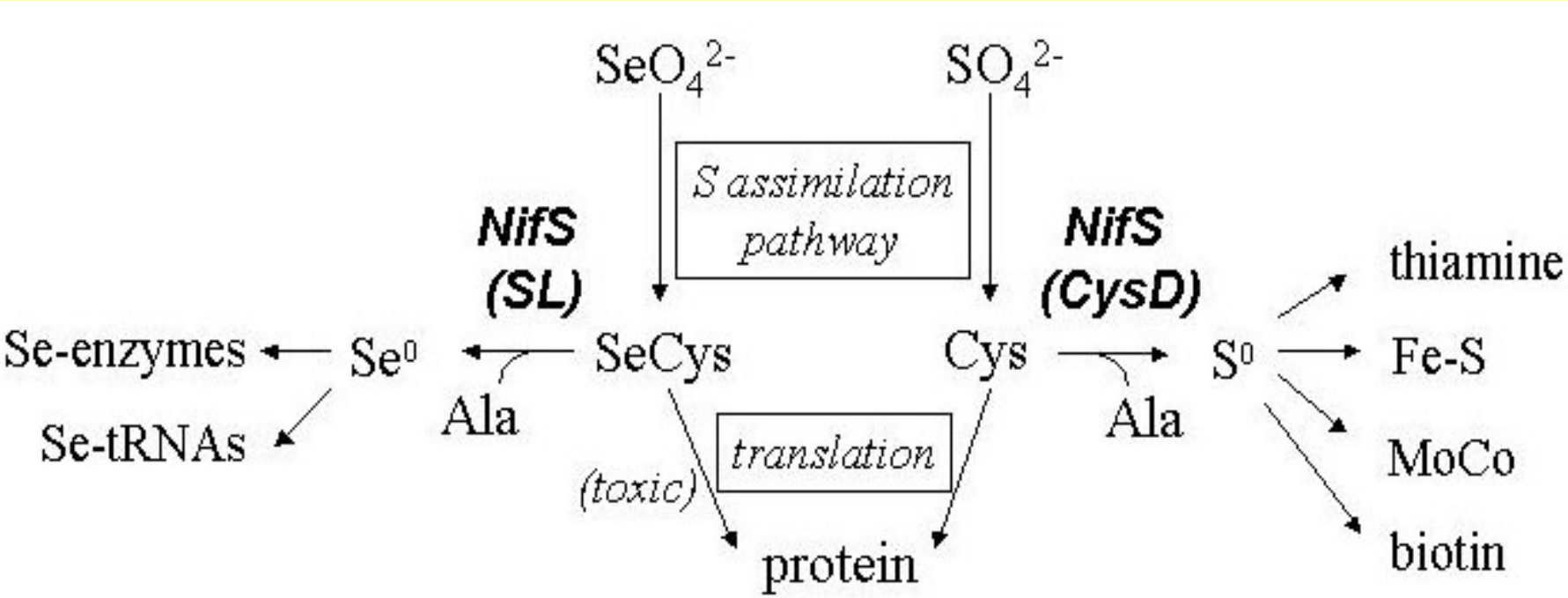


Arabidopsis thaliana cpNifS

- In chloroplast
- SeCys Lyase activity and Cys desulfurase act

300-fold higher

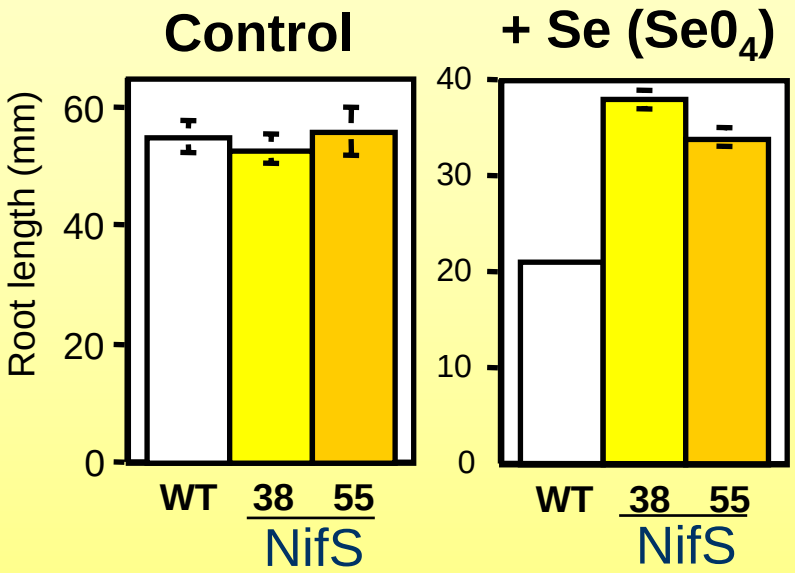
Functions?



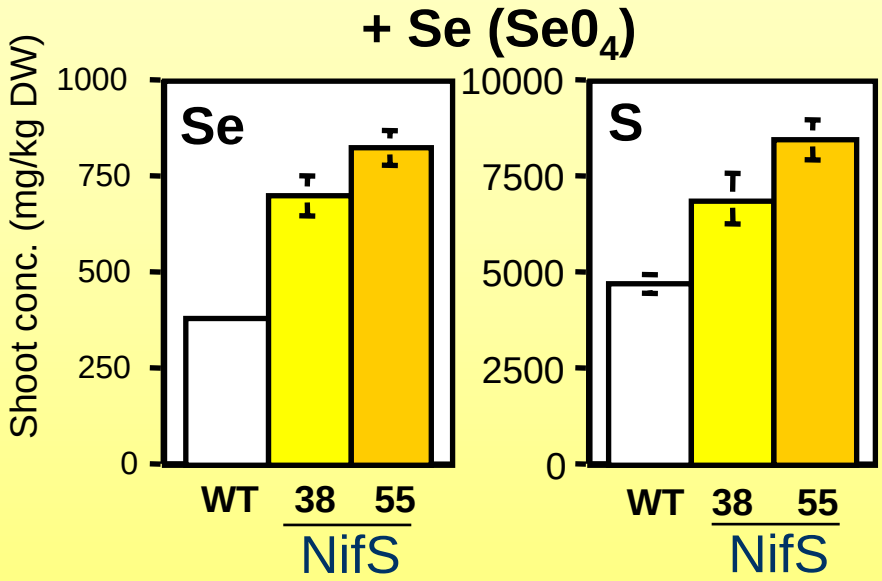
CpNifS overexpression

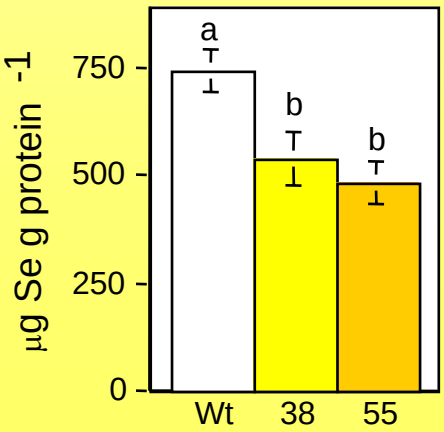
Expression (Western blot)

enhances Se tolerance…



…and Se & S accumulation





Less Se incorporation into protein

Function in Se tolerance?

Microarray analysis

Material

Wildtype & CpNifS overexpressors

Treatments

+/- 40 μM selenate

Findings

General:

Effect of Se on transcriptome indicative of stress and S deficiency

Effects *less/more pronounced in overexpressors:*

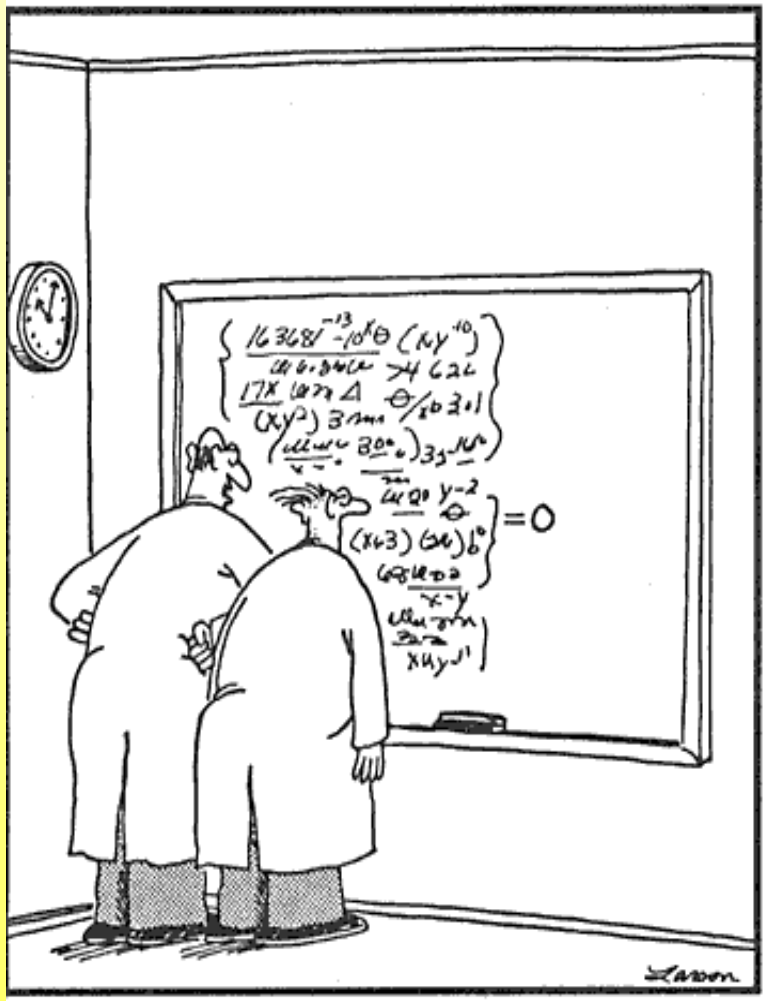
- Genes related to S-deficiency less upregulated, but sulfur transporter sultr2;1 (root xylem loading) more upregulated
  → explains higher S, Se in shoot
- General stress proteins (e.g. hsp) expressed, induced more in overexpressors

Conclusions – Part I

It is possible to genetically manipulate plant Se metabolism

This may be used to:

- enhance phytoremediation efficiency
- create fortified foods
- promote basic scientific knowledge



"No doubt about it, Ellington—we've mathematically expressed the purpose of the universe. God, how I love the thrill of scientific discovery!"

Ecological Aspects of Plant Se Accumulation

How does Se accumulation affect other organisms?

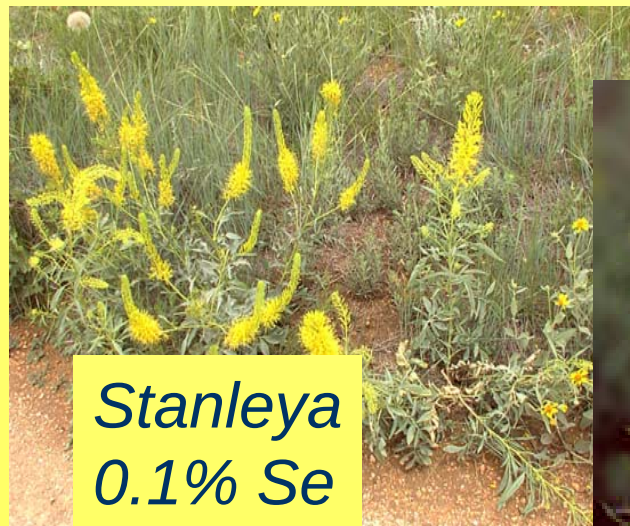
herbivores
microbes
plants

Brassica juncea

Astragalus
0.5% Se

Why do some plants hyperaccumulate Se?

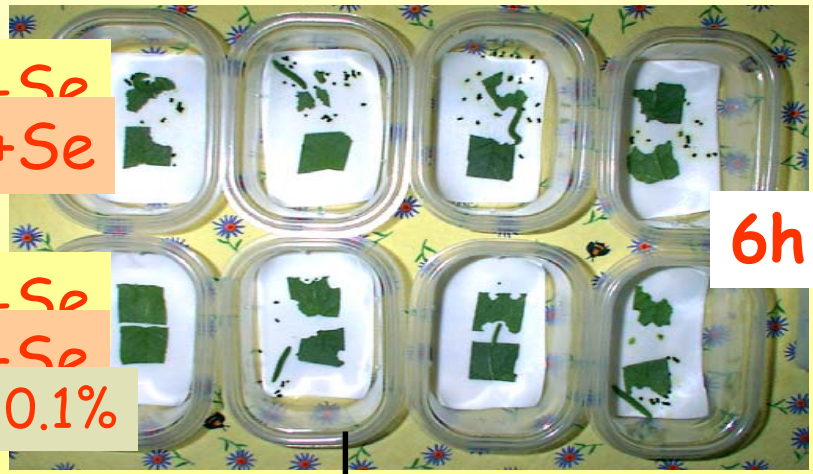
Does Se protect against biotic or abiotic stress?

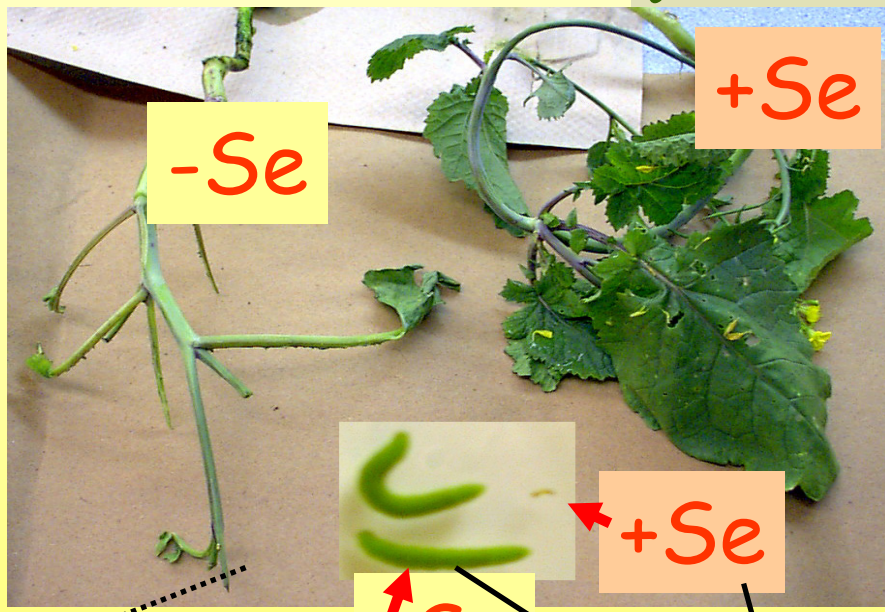


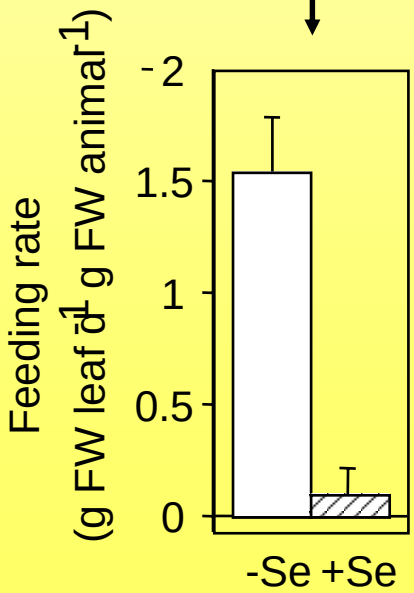
Stanleya
0.1% Se

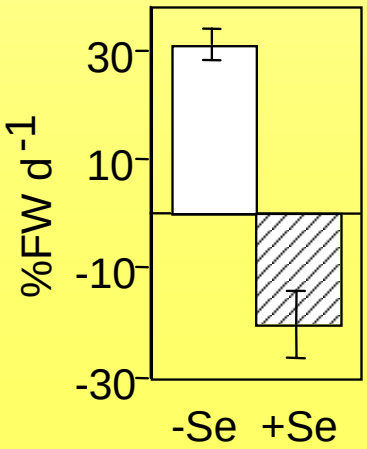
Pieris rapae caterpillars prefer Brassica leaves -Se

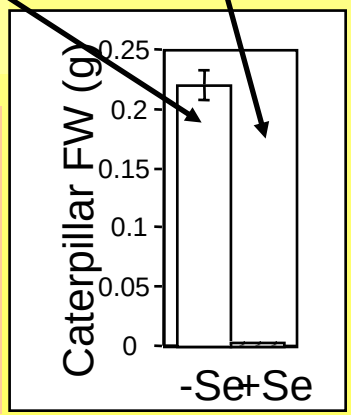
Brassica juncea





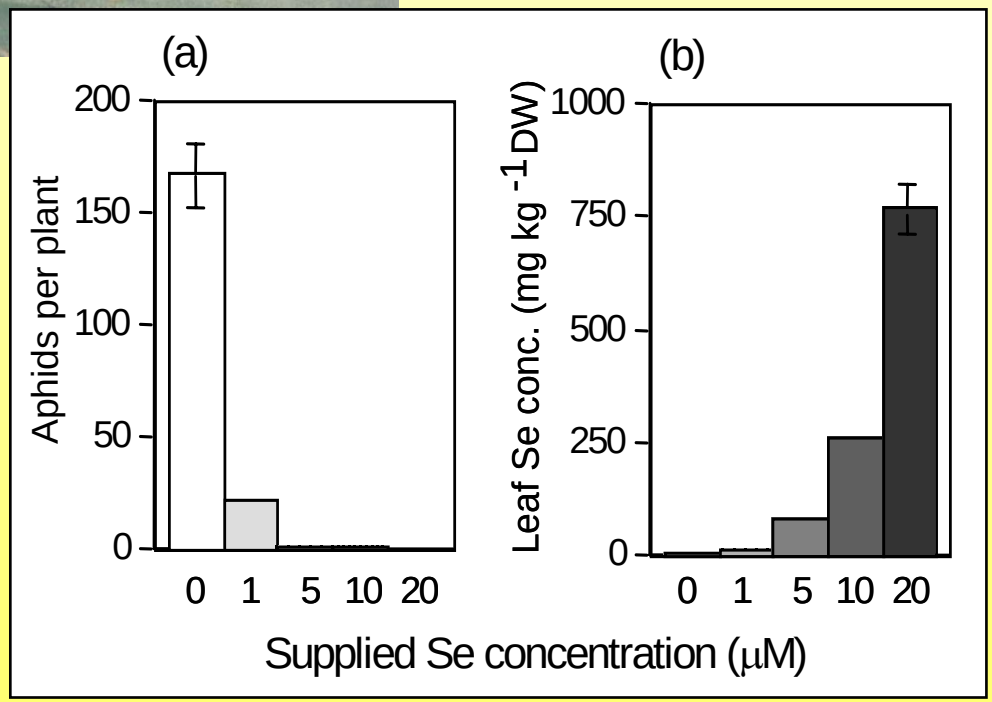


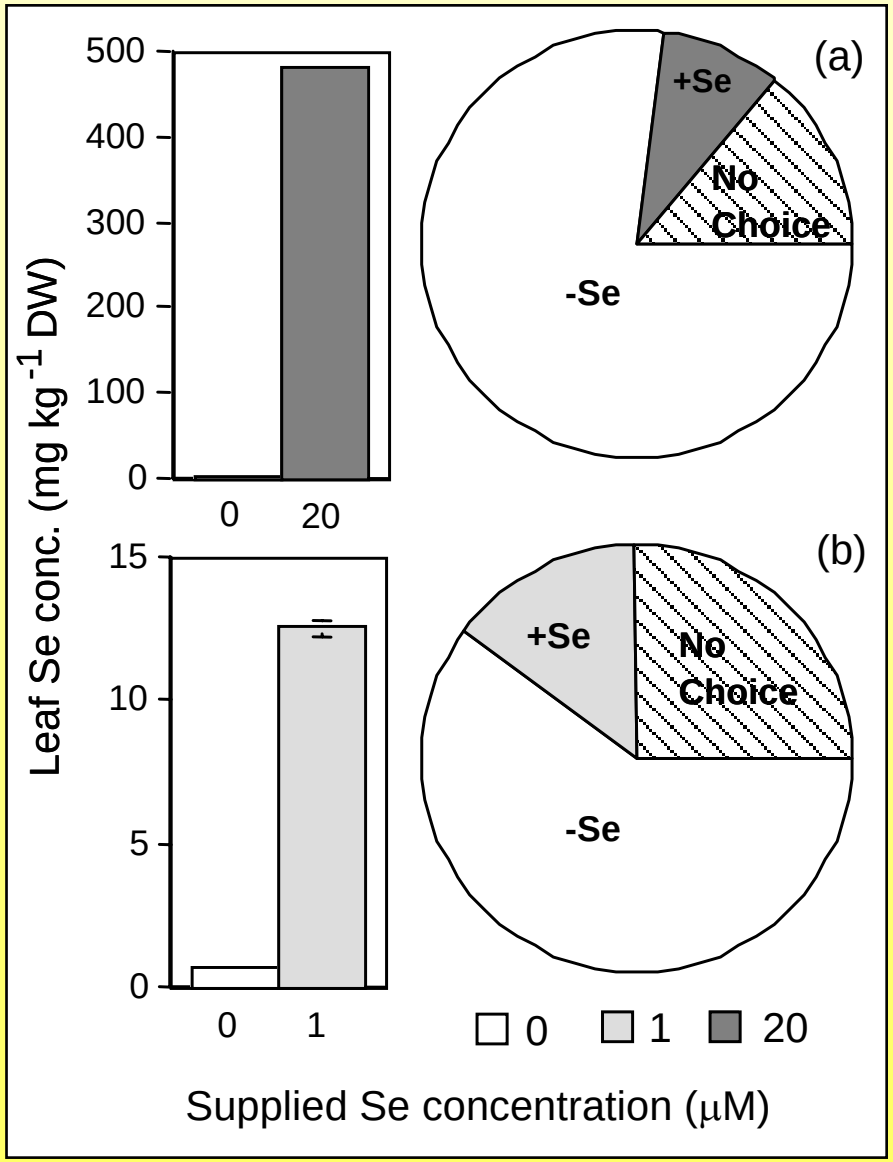


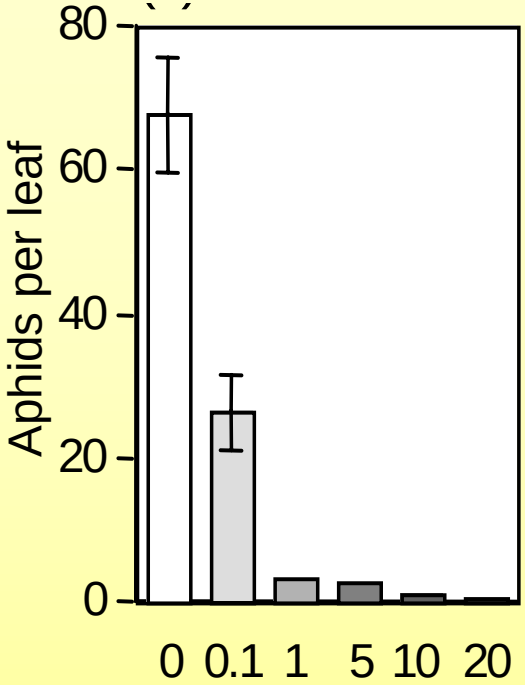


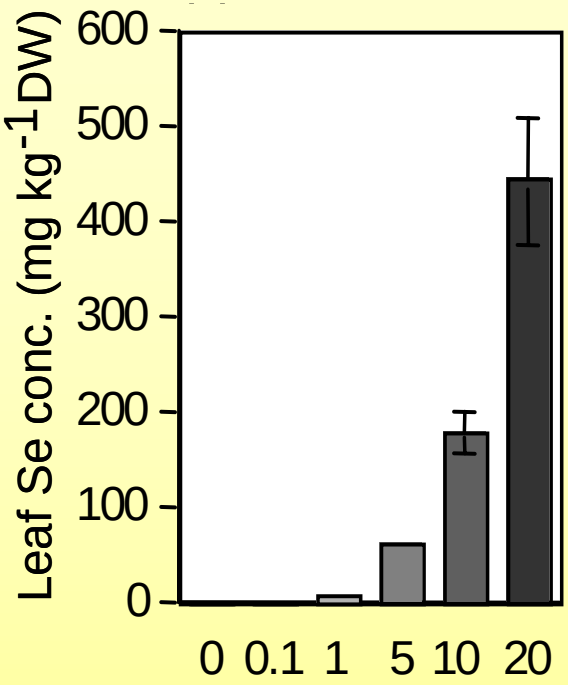
+Se plants are toxic to the caterpillars

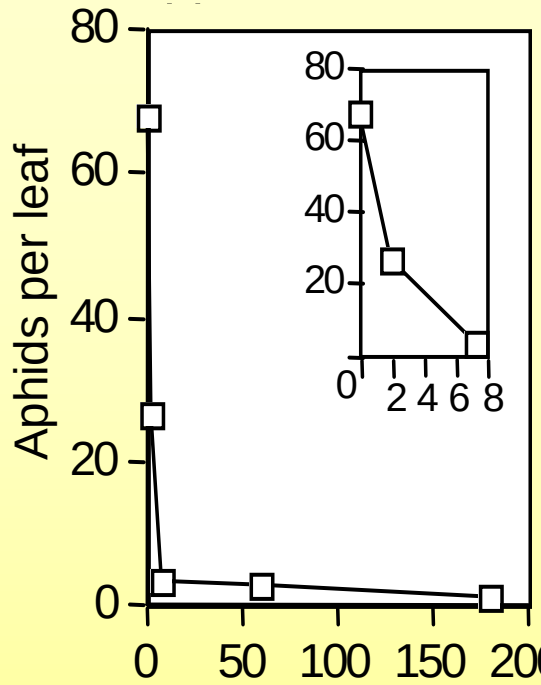
Se in leaves deters colonization of Brassica by green peach aphids



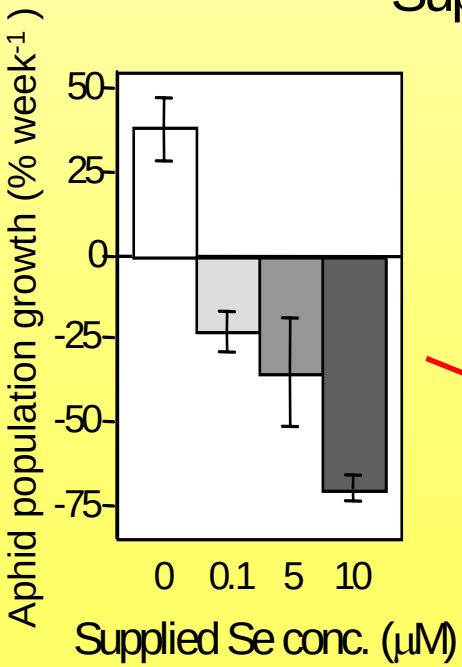








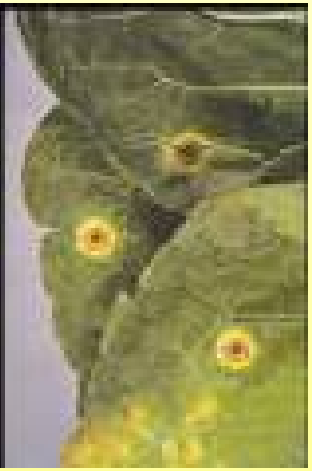
Supplied Se concentration (µM)



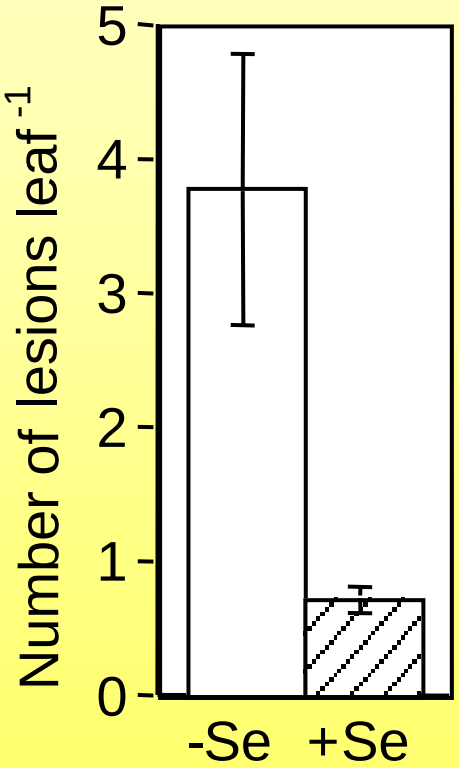
Se in leaves is toxic to aphids

... can be used as a pesticide

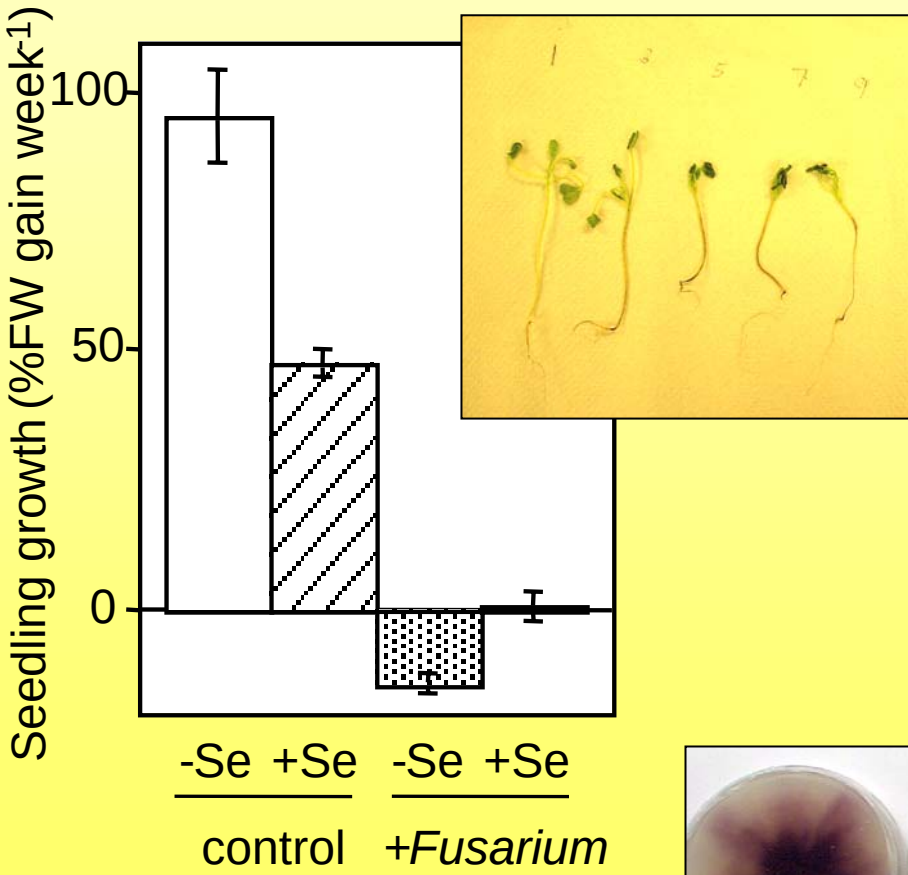
Se protects *B. juncea* from fungal infection



Alternaria

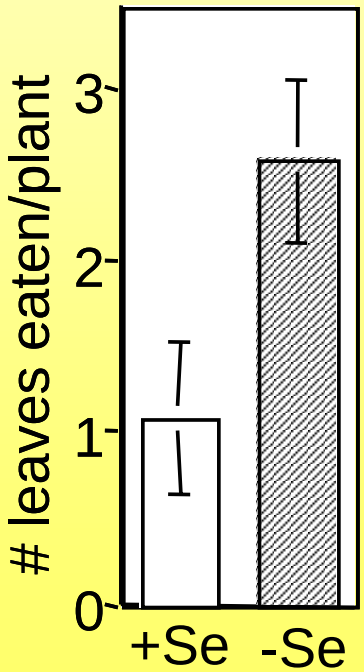


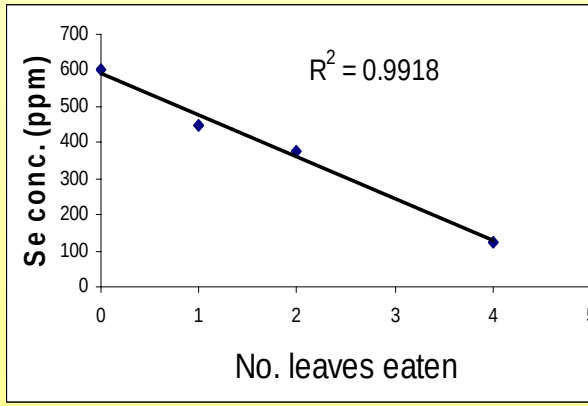
Fusarium



Field study using plants +/- Se

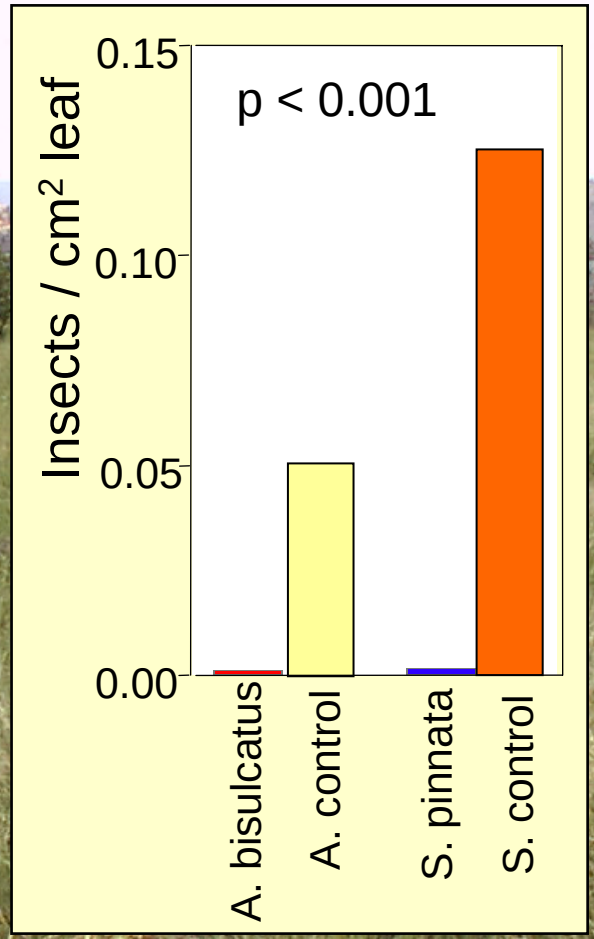
Se may protect plants from prairie dog herbivory





B. juncea

Se hyperaccumulators in the field contain fewer insects per leaf area than nonaccumulator relatives



Hyperaccumulator species appear to harbor specialist herbivores

Conclusions – Part II

- Se can protect plants from herbivory
- Se can protect plants from fungal infection

→ sheds light on possible selection pressures for evolution of hyperaccumulation

→ may have implications for phytoremediation and applications in agriculture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