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Viral infection and nicotine conversion to nornicotine in the next generation.

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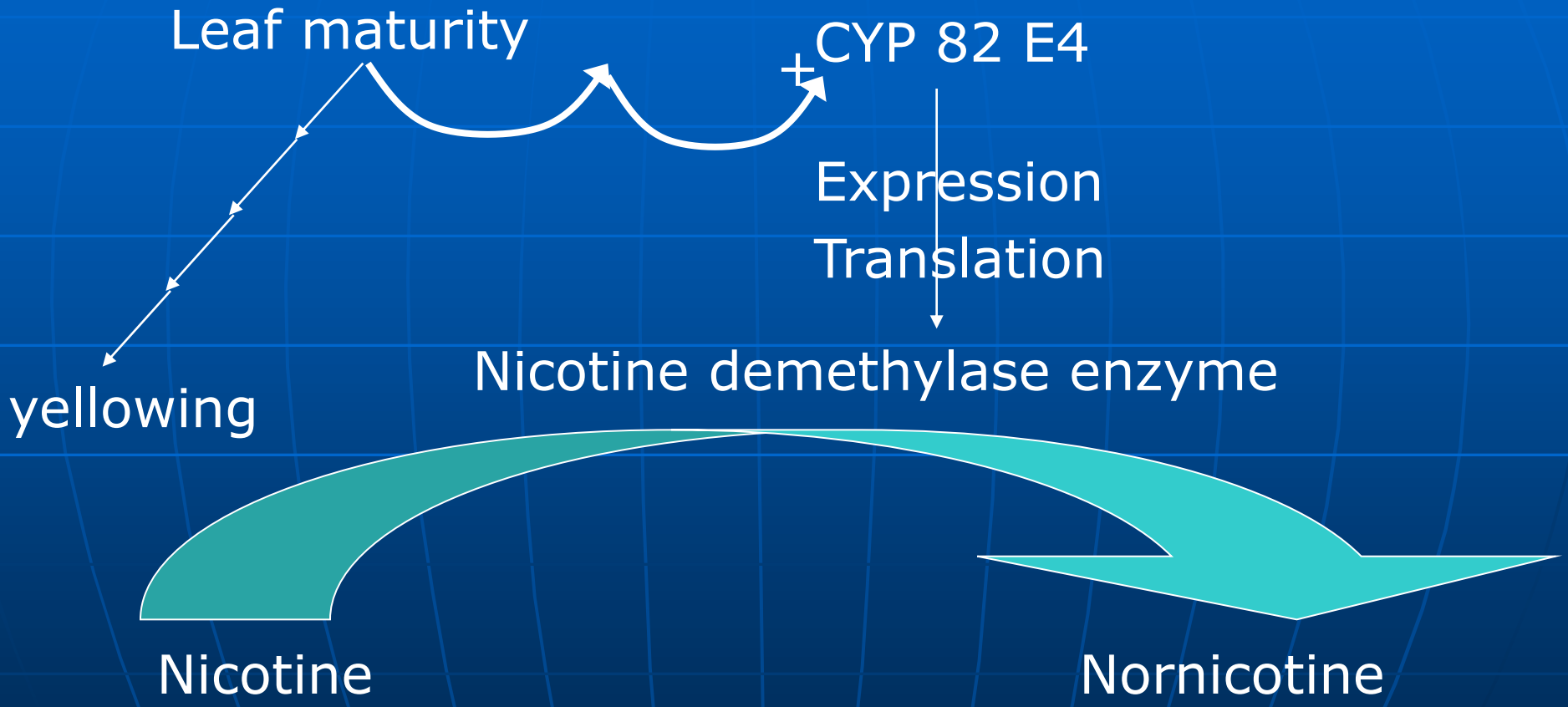
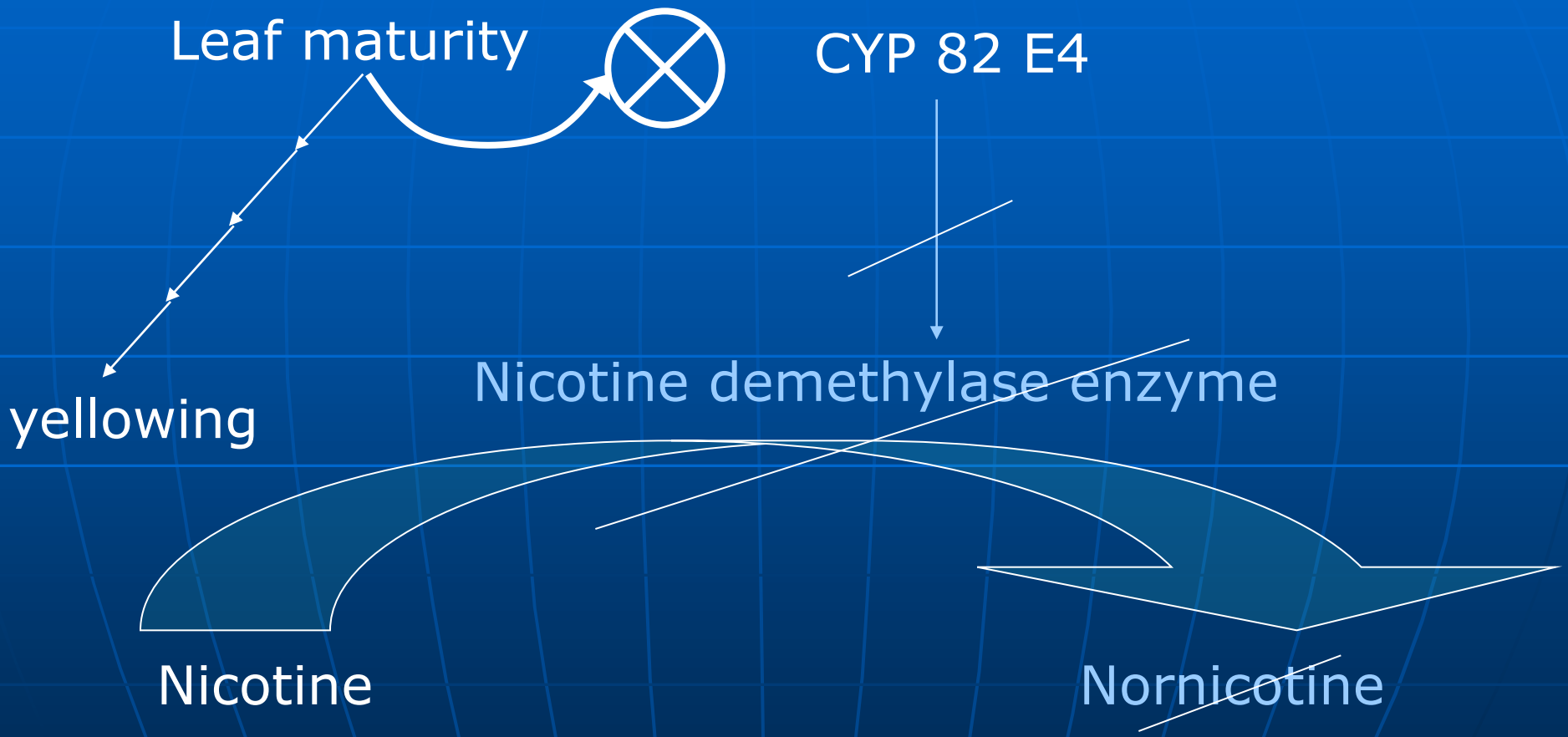
Nicotine conversion to nornicotine

- May occur during leaf yellowing & curing.
- Converter plants may be found in the descent of non converter plants
  - More frequent in burley.
  - The nicotine demethylase gene is present in any plant, converter or not.
  - In non converters, its expression is blocked
    - Epigenetic event(s) would allow the expression of the nicotine demethylase gene in part of the next generation?
    - What is the cause of such events?

Converter tobaco



Non converter tobaco



What would be such epigenetic change and how would it be triggered?

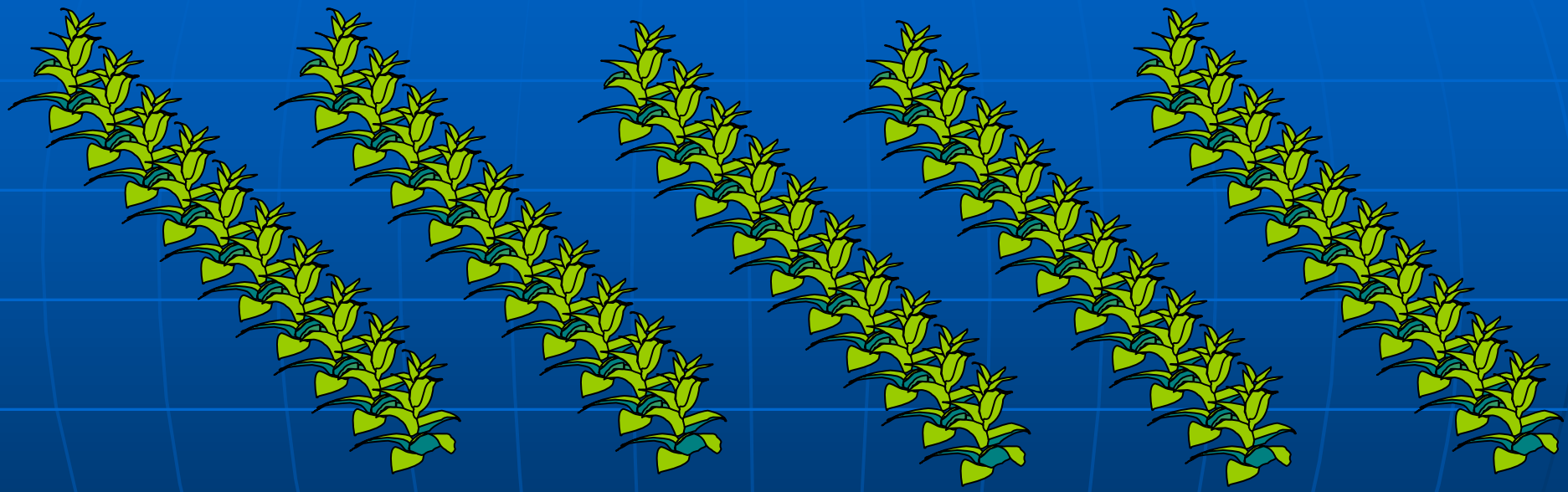
- DNA recombination?
  - The progeny of TMV-infected plants exhibited an over 3 fold increase of inherited changes in the luciferase transgene
    - Kolwalchuk I. et al., 2003
- DNA methylation?
  - TYLC Sardinia virus infection on tomato led to DNA methylation of genes involved in virus defense response.
    - Mason G. et al., 2008.
- *Virus infection? Other stresses? ?*

The experiment

- 2006: producing progenies from the burley TN 90LC virus infected / non infected plants
- 2008 and 2010
  - Field tests in an attempt to estimating the frequencies of converter plants in each kind of progeny

TN 90 LC

No inoculation | Water =Mock | CMV | PVY | PVY+CMV



- Bergerac 2006
- Inoculated in the field 2 weeks after transplantation
- PVY^{N}, CMV DTL
- Symptoms + ELISA tests confirmed inoculation success

Self pollinated 10 shoots / treatment

No inoculation | Water =Mock | CMV | PVY | PVY+CMV

- Harvested and cleaned separately the seeds
  - from each individual shoot
  - in each treatment

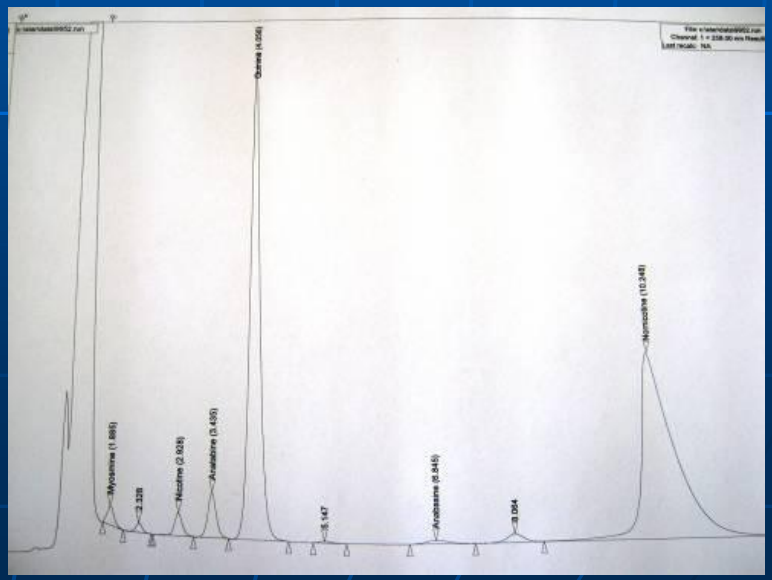
Bulks

- PVY seed bulk
  - Equal amount of seeds from each of the 10 PVY^N inoculated shoots
    - Bulked → next generation « TN90 PVY »
- Same for CMV, CMV + PVY, mock and non inoculated

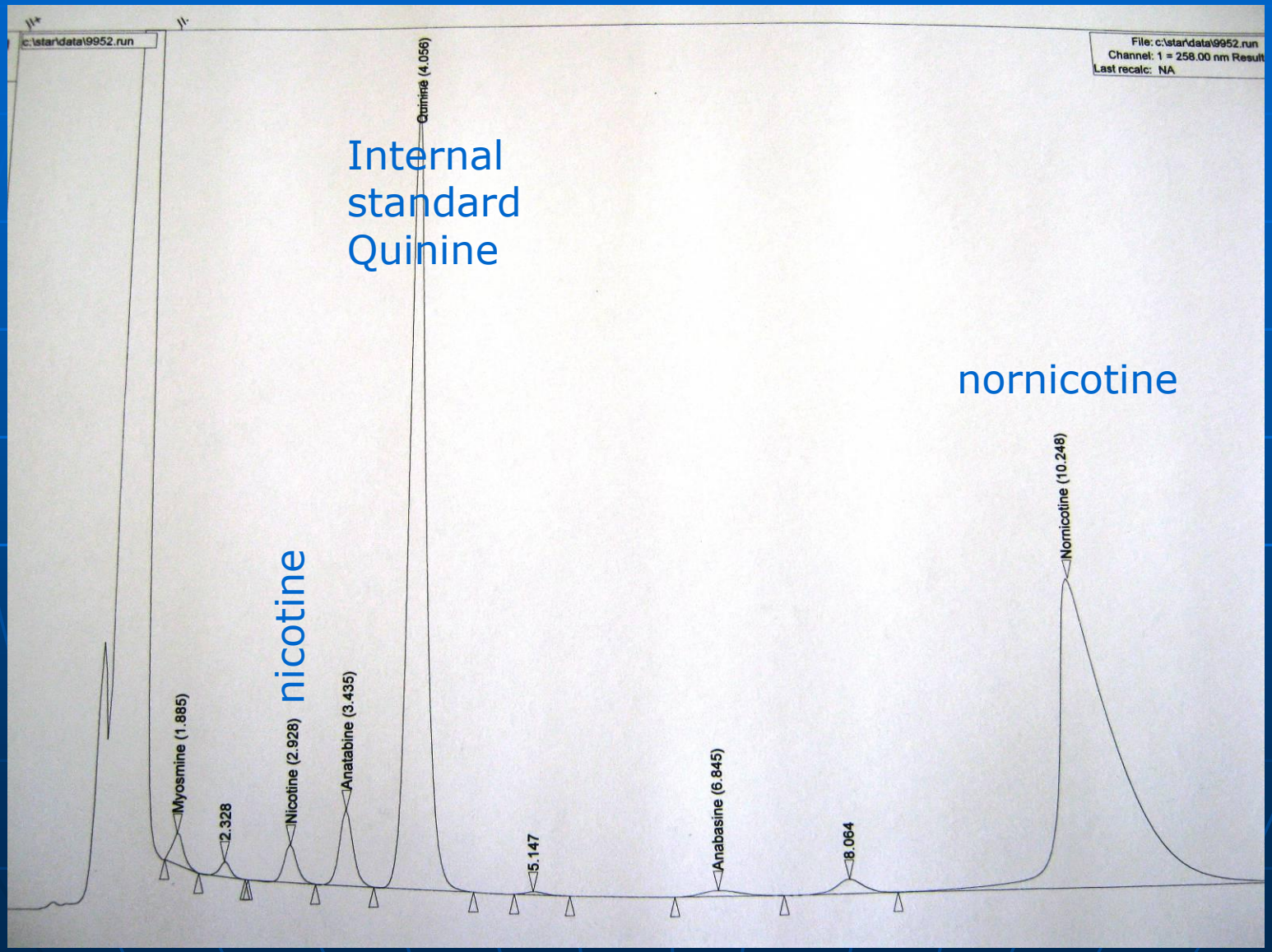
Studying bulked progenies

- Tranplantation: May, grown as usual, untopped
- At maturity (September), harvested 2 middle leaves on each shoot
  - Air cured
  - Yellowing as complete as possible
  - Ground to powder (whole leaf)
  - Alkaloid extraction
  - HPLC analysis

Estimating nicotine conversion



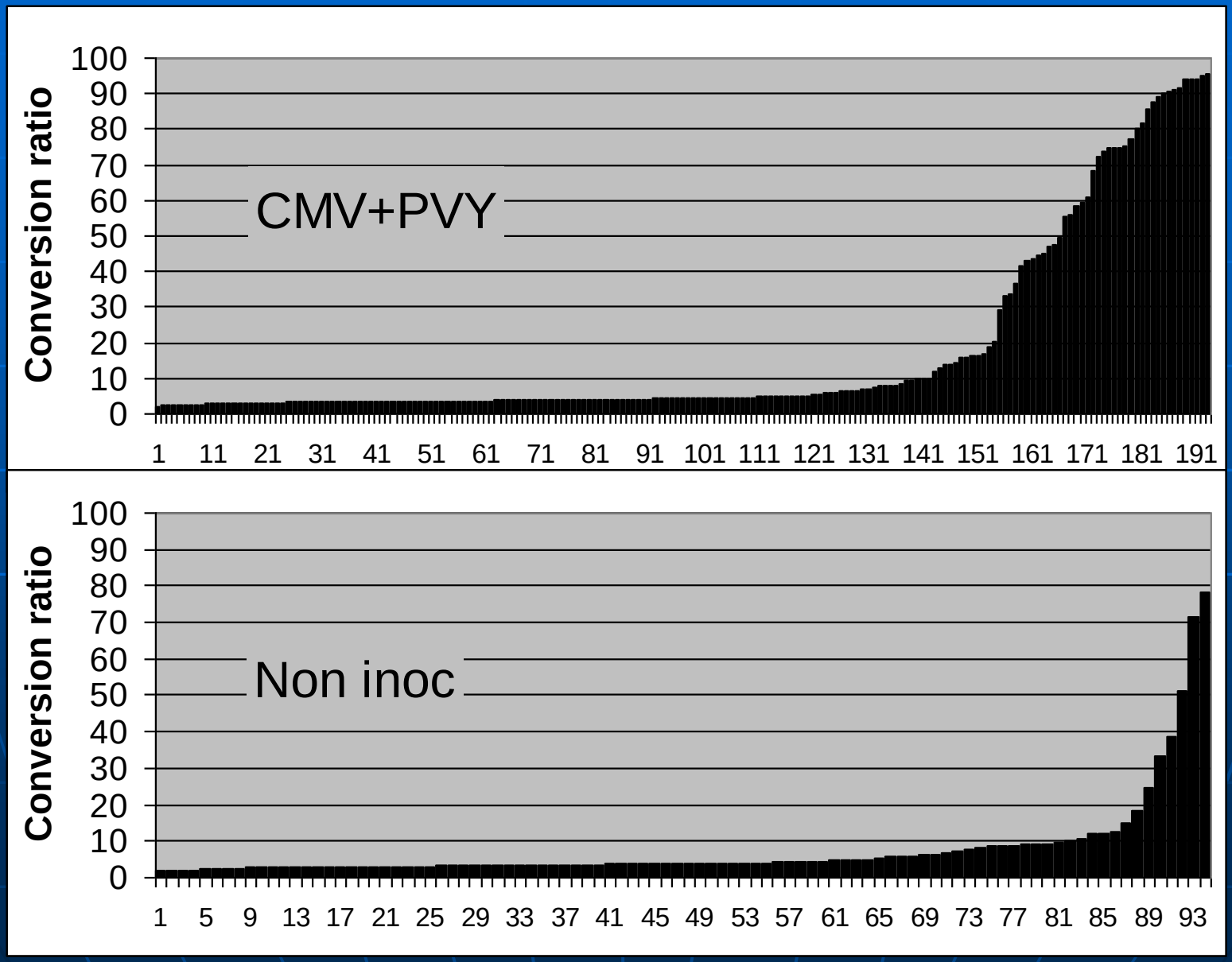
HPLC, converter plant



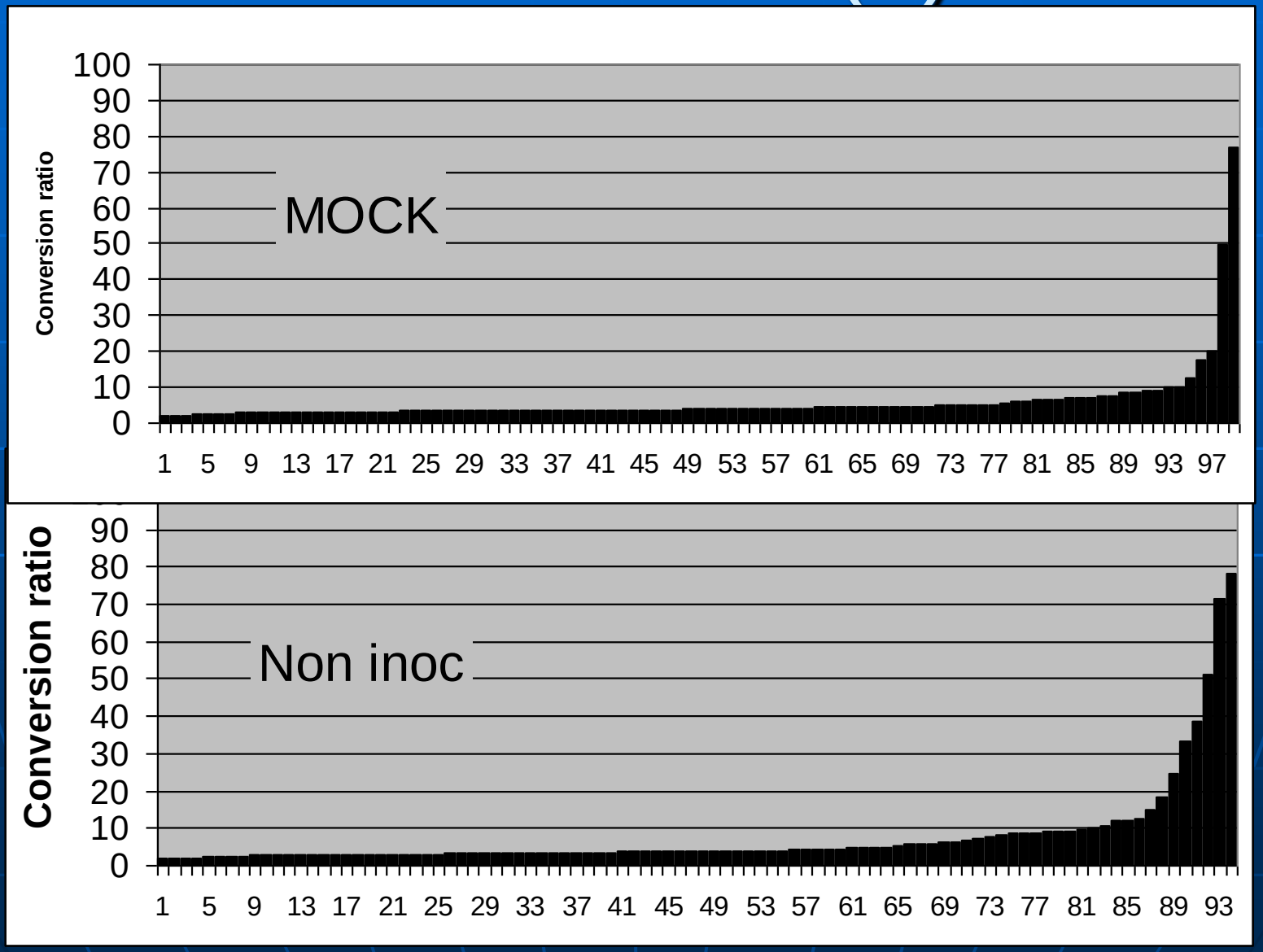
Number of plants studied / bulk

	Non inoculated	Mock	CMV	PVY	CMV + PVY
2008	100	100			200
2010		50	100	100	

2008 results



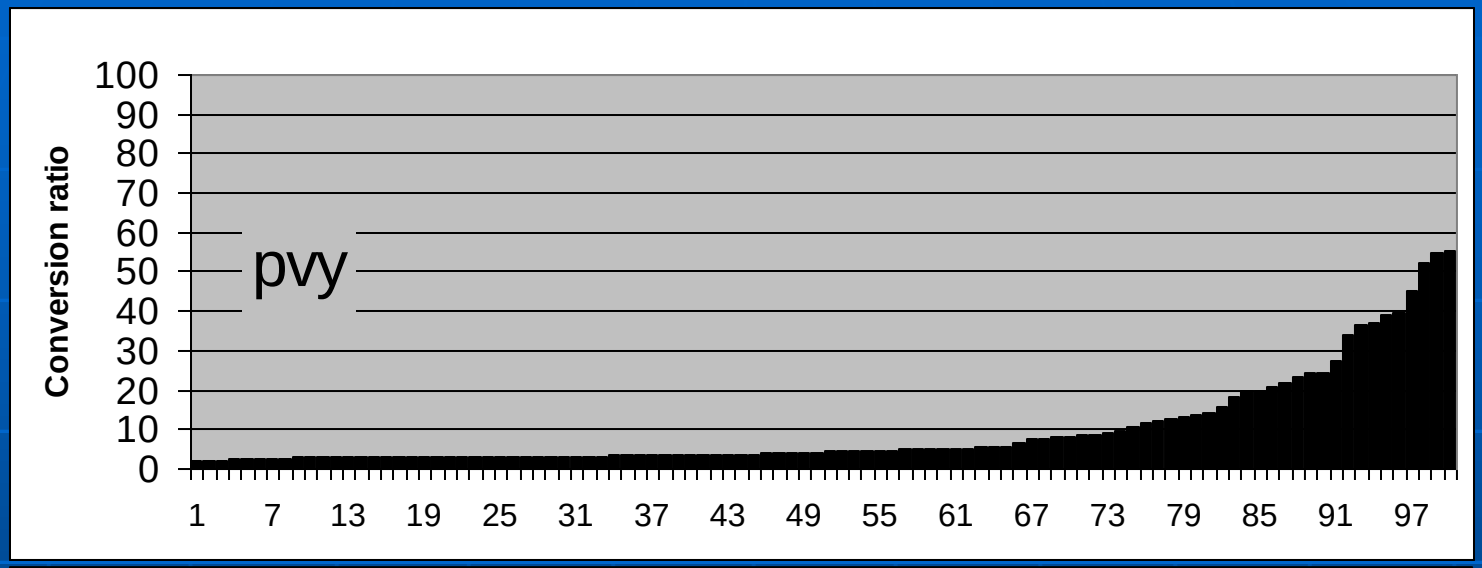
2008 result (2)

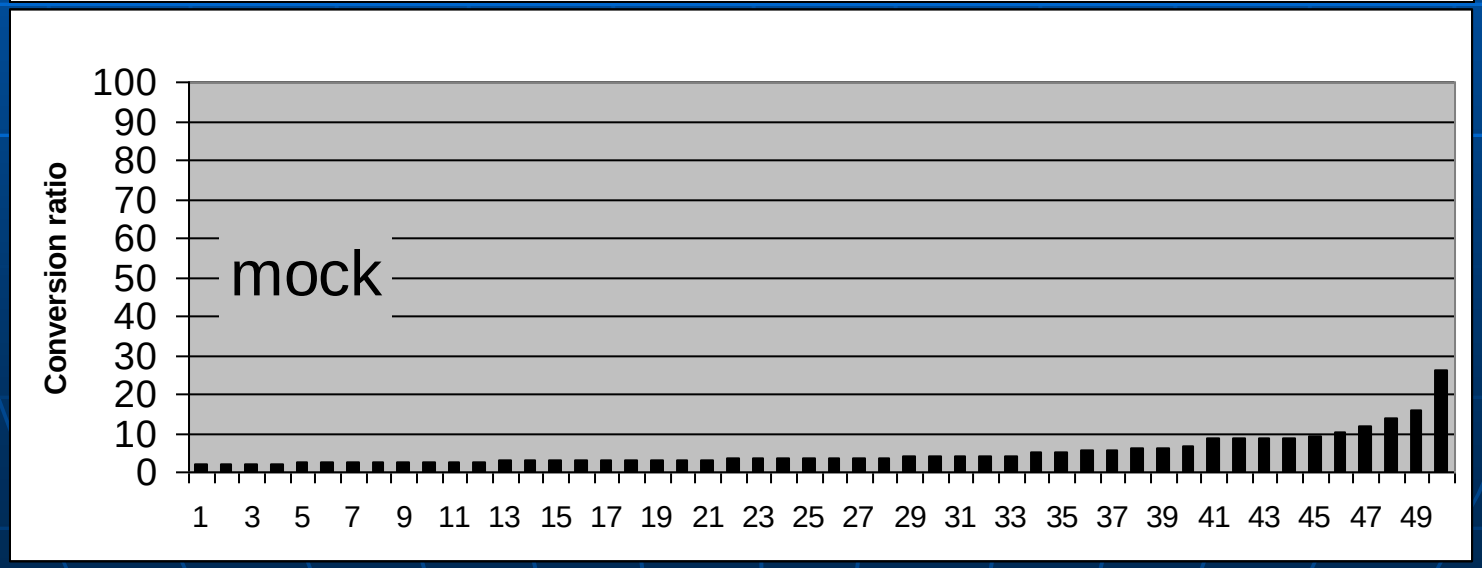


2008 results – stat.

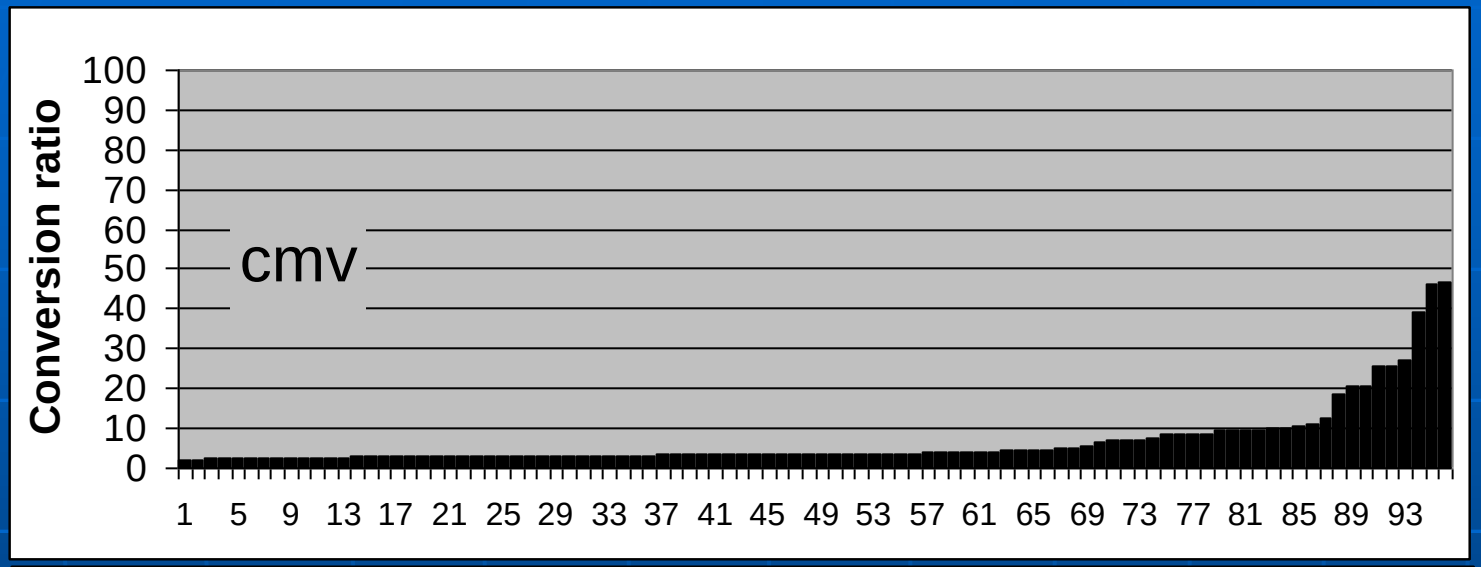
	total shoots analysed	Frequency of converters (ratio > 10%)	Frequency of converters (ratio > 15%)
Non inoculated	94	0,14	0,07
Mock	99	0,06	0,04
CMV+PVY	193	0,26	0,24
Red= significant difference from non inoculated (5%)			

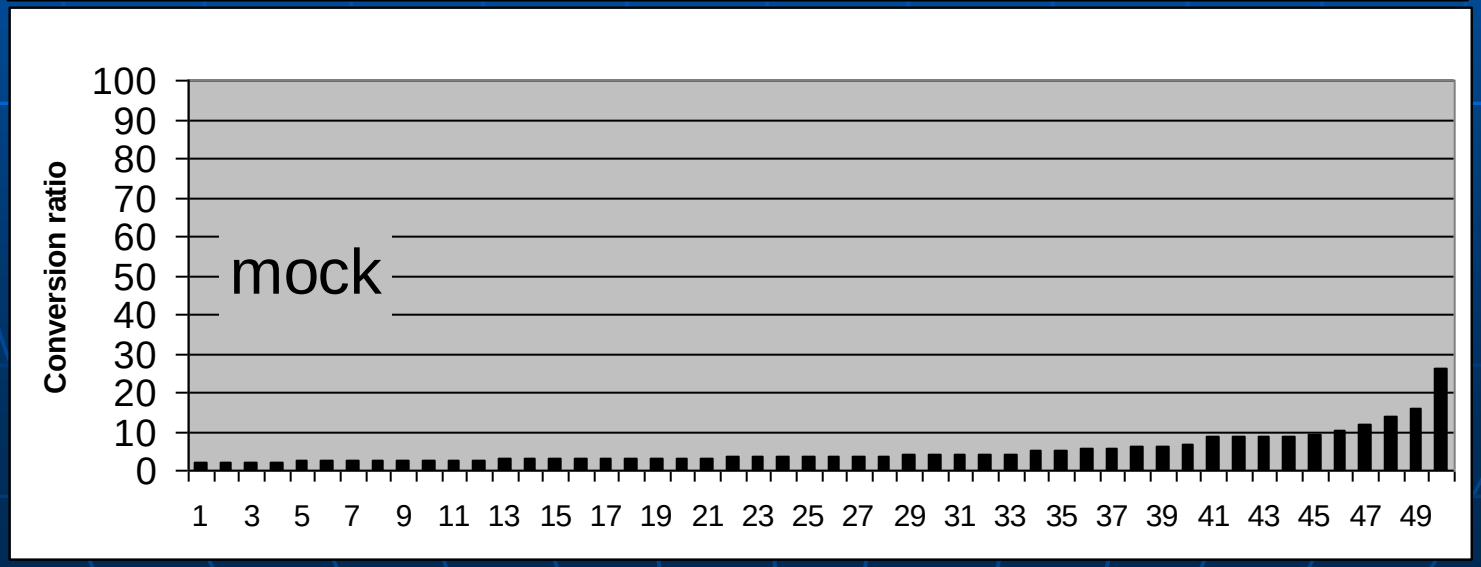
2010 results -1





2010 results - 2





2010 results – stat.

	total shoots analysed	Frequency of converters (ratio > 10%)	Frequency of converters (ratio > 15%)
Mock	50	0,10	0,04
CMV	96	0,15	0,09
PVY	100	0,26	0,19
Red= significant difference from Mock (5%)			

Conclusion

- Viral infections significantly increased the frequency of converter plants in progenies.
  - CMV+PVY: 2 – 3 fold
  - PVY: 2 fold
  - CMV: 1.5 fold (ns).
- Discard virus infected plants in seed production
  - As soon as possible
  - Foundation seeds and seed production

Perspectives

- Use of epigenetic induced changes in breeding?
  - May well be already the case in a non intended, non conscious way.
  - If conscious, could be optimised
    - Suggested in Mason et al. 2008: molecular tools to identify methylated genes
    - Review: Boyko & Kovalchuk 2010
- Maintenance of true-to-type inbred lines: avoid epigenetic changes