

Environmental Risks from Transgenic Crops

Jonathan Gressel

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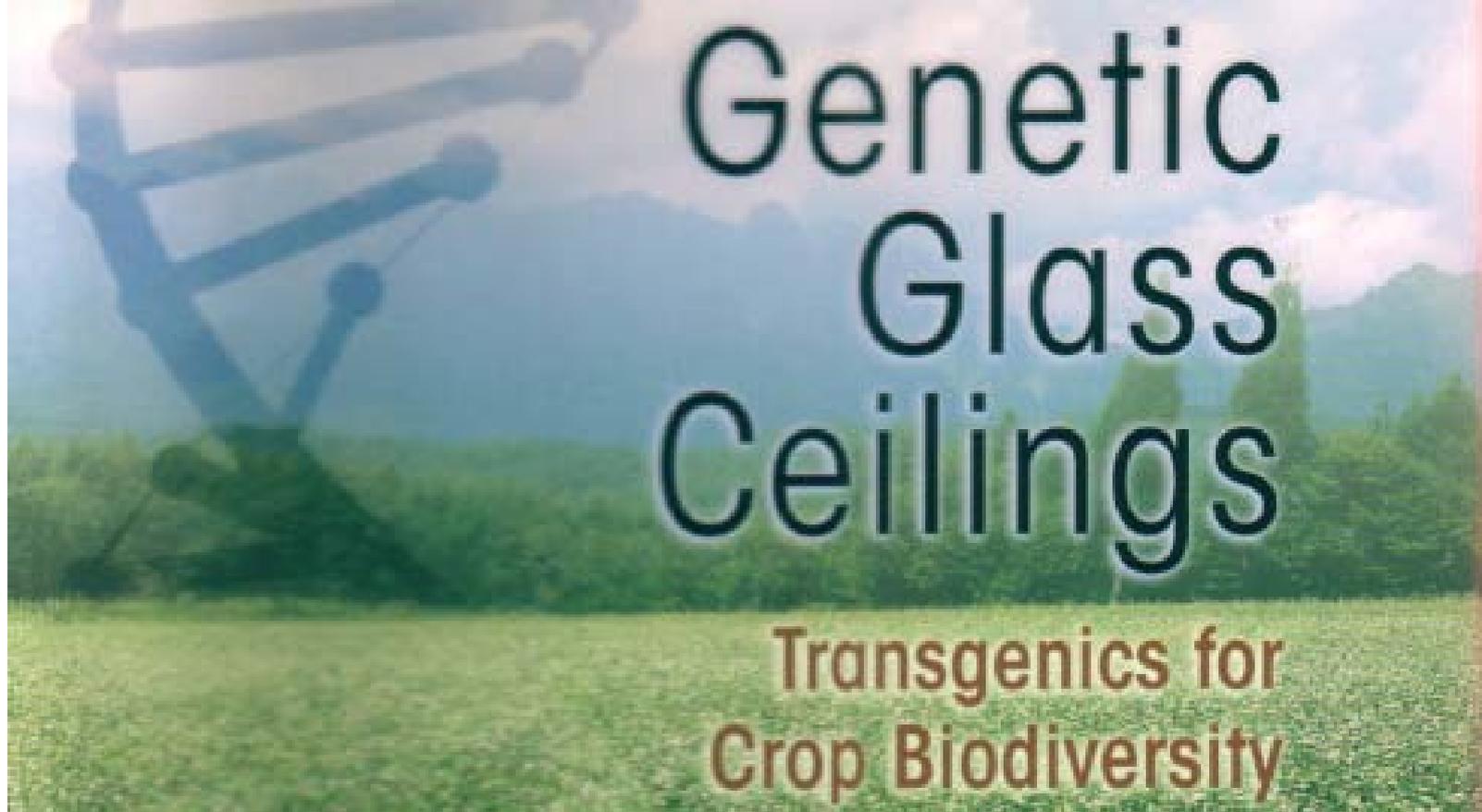
מכון ויצמן למדע

WEIZMANN INSTITUTE OF SCIENCE

Rehovot, Israel

Pontifical Academy of Sciences
May 18, 2009

A new
book



(Johns Hopkins Univ. Press - 2008)

deals with further domesticating under-
domesticated crops that lack genetic diversity
Analyze and proposes where to get the genes
Developing world has many such crops

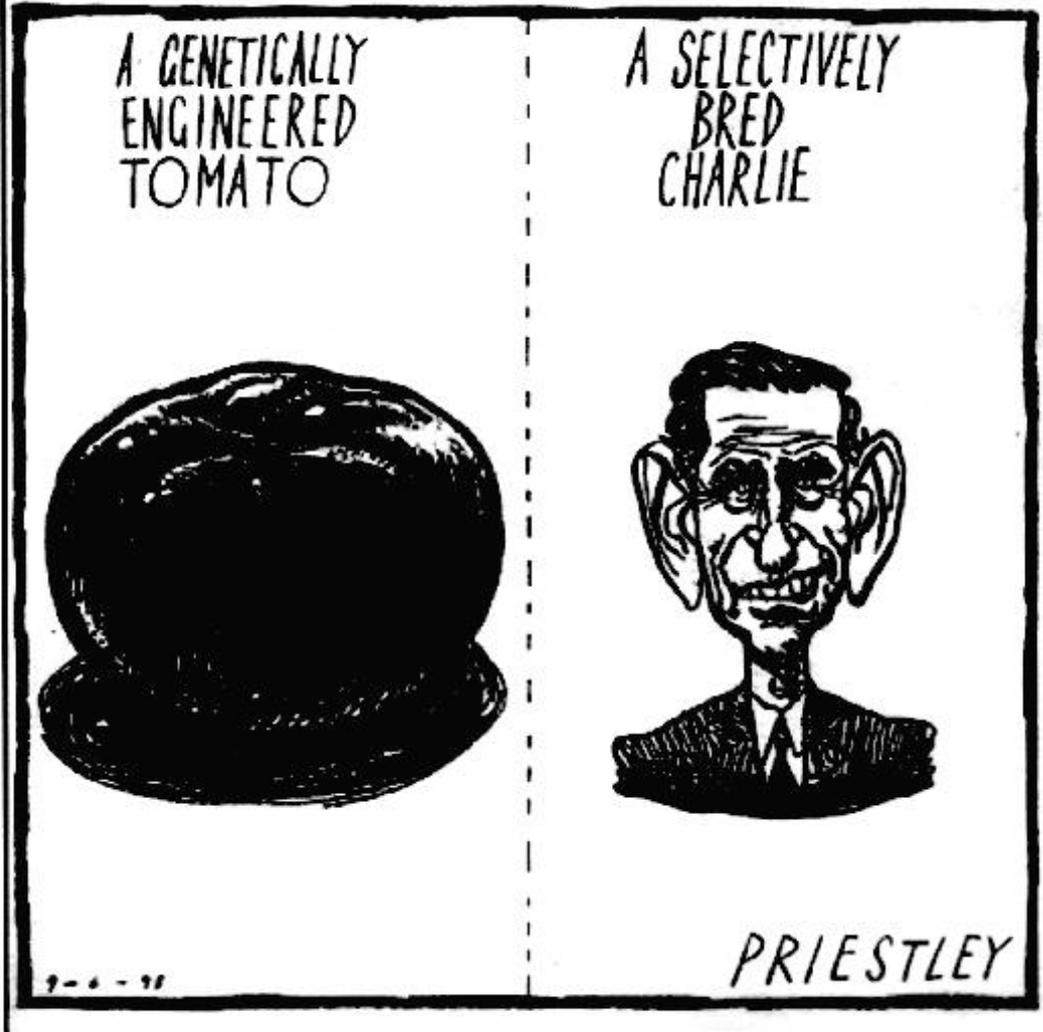
Transgenics allow rapid domestication

Bring in genes from wherever

Activate /suppress specific genes whenever

THE INDEPENDENT

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THE INDEPENDENT ON THE INTERNET: WWW.INDEPENDENT.CO.UK



Genetic Engineering vs. Inbreeding Depression

Problems in Africa

HIV

Witches



These are
beautiful plants

HIV=Highly
Invasive
Vegetation

Photo: Lytton Musselman



that were never controlled until they emerged from the soil, which is too late.

Striga hermonthica
(witchweed) on
sorghum in Africa

Photo: Lytton Musselman



Photo: Lytton Musselman

Striga (witchweed) bewitches maize as well

Human population displacements facilitate disease epidemics

- war
- labor migration
- land abandonment

HIV=Highly Invasive Vegetation

Causes:

- labor migration
- land abandonment

Crop production in Africa

- partially the effect of *Striga*

	Sorghum	Maize
--	---------	-------

	Yield (T/Ha)	
--	--------------	--

World	1.4	5.0
-------	-----	-----

Sub-Saharan Africa	1.0	1.8
--------------------	-----	-----

USA	4.7	9.4
-----	-----	-----

Data for 2007, from FAOStat

Parasitic weeds germinate after stimulation by crop roots, and grow towards the crop roots, attach, and suck nutrients

Striga also poisons the crop.

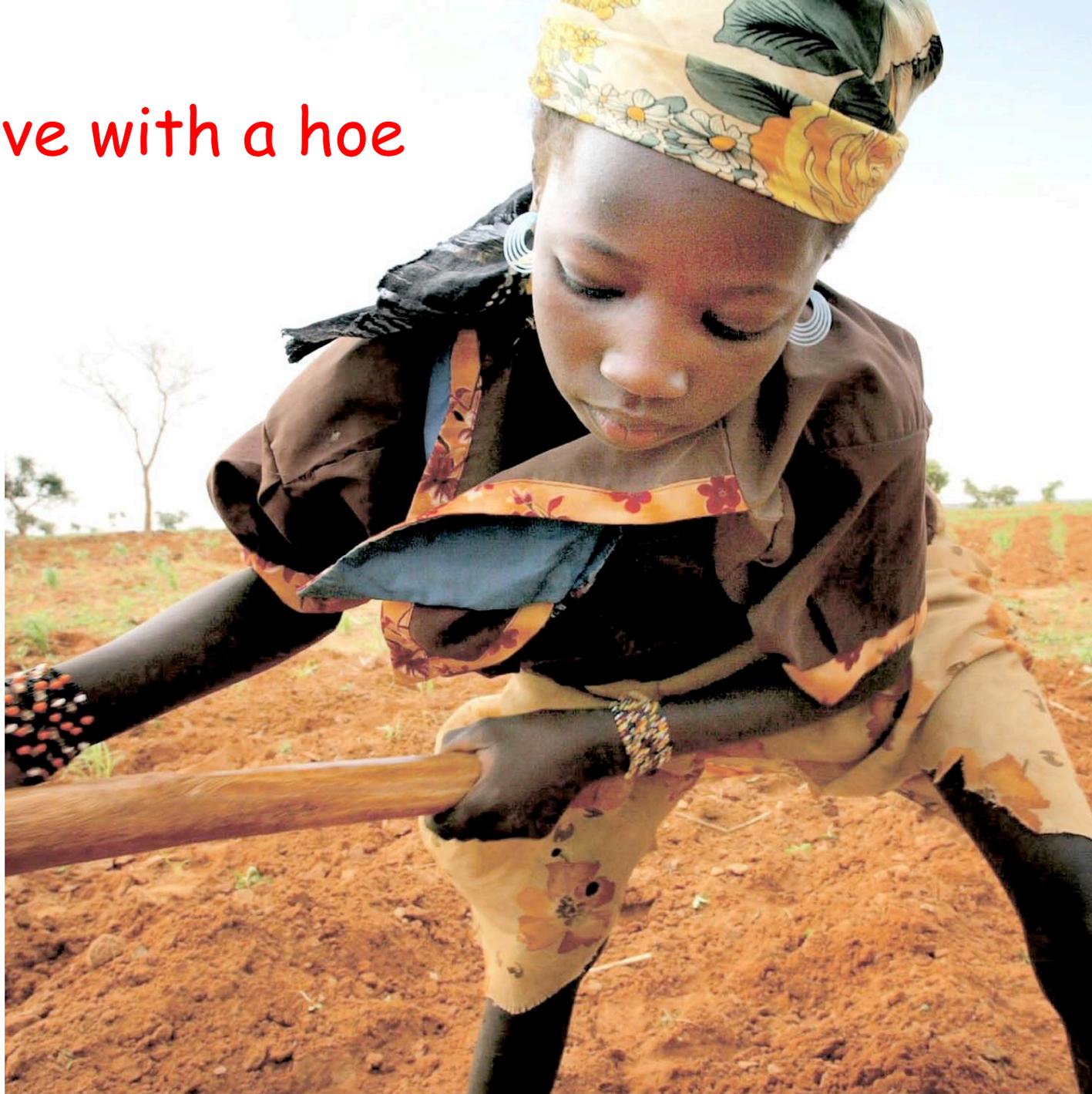




Sucks

Can't remove by hoeing!

Cannot remove with a hoe



A theory was formulated -

-The herbicide should move unmetabolized from the leaf, through the stem into the parasite, underground





Orobanche spp similarly attack other crops

It took some convincing to get industry to provide the genetic material for testing



+chlorsulfuron



ALS Resistant tobacco

+ glyphosate



EPSPS Resistant oilseed rape

How could this work for *Striga*?

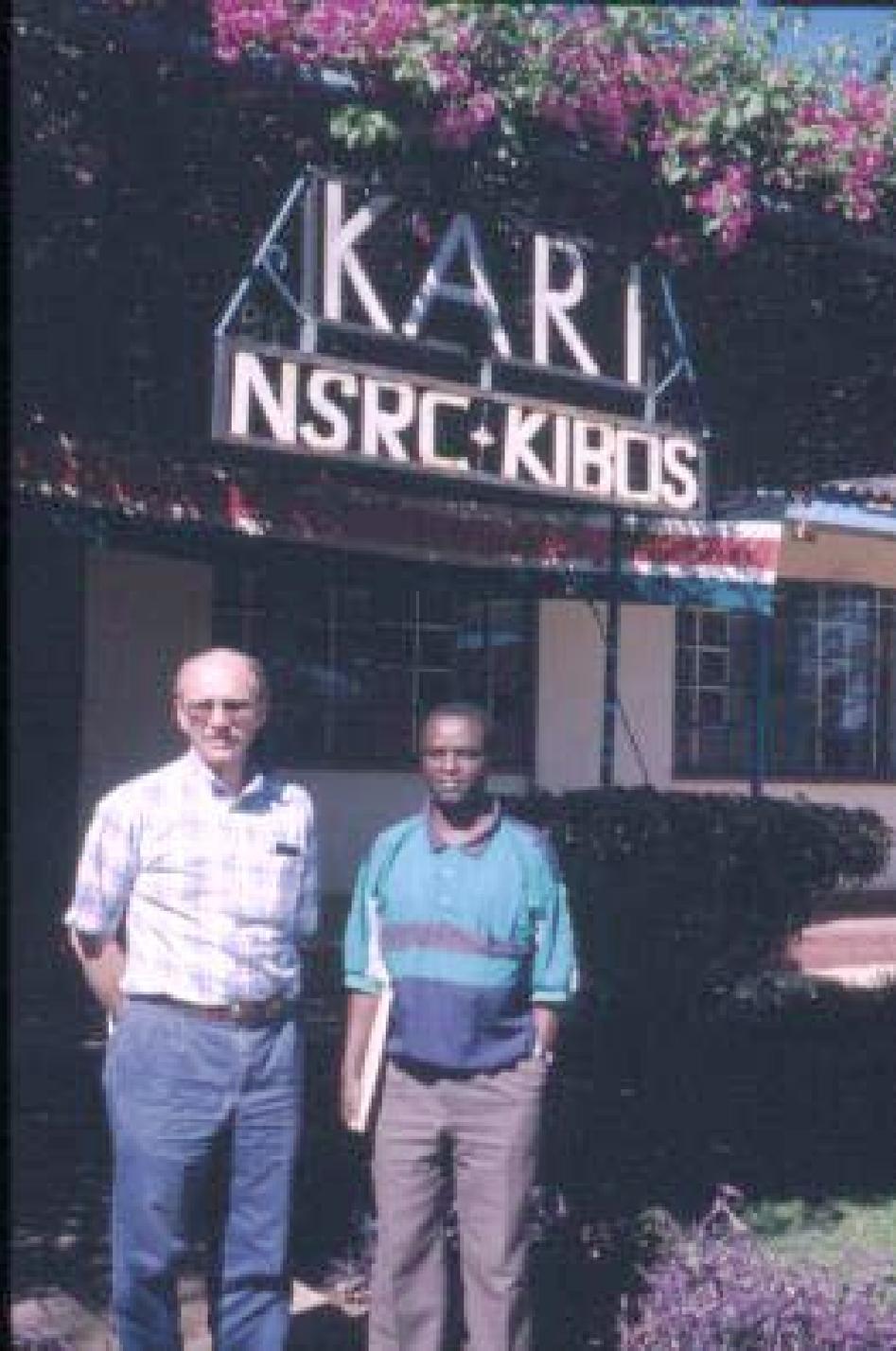
• *farmers can't afford herbicides
to spray over whole fields*

- farmers can't afford sprayers

Approach:

Treat crop seeds with herbicides instead of fields

Need target-site resistant crops having a very large margin of resistance.



Joel Ransom

Fred
Kanampiu

IR - maize (homozygous)

ALS try₅₅₂ \Rightarrow leu

Pioneer - AmCy (BASF)

A mutant obtained through biotech



LOCAL CHECK
H513
PLANTED 15/3/02

IR MA...
SINGLE CROSS-1
PLANTED 15/3/02

Kanampiu

Diallo

Photo: Dennis Friesen



Photo: Dennis Friesen

Technology for witchweed commercialized
for maize through local seed companies

But not sorghum - although at least three
groups now trying to get same mutant in...
genetically - there is a good reason why
transgenic better than mutant selection

Better technologies coming on line:

e.g.

Dan Kiambi (Nairobi) RNAi in crop for *Striga*
specific genes



Jane Rissler:

"Herbicide resistant crops are
.....a capitalist conspiracy.....
(and later)
..... a male chauvinist conspiracy"

Florence Wambugo:

"A women's place is in the lab engineering
herbicide resistant crops so other women
should not have to weed by hand"

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European Corn Borer

16M ha infested in U.S.
1-3 borer generations per year
Mean 6% yield loss; up to 30%

Lep pests worldwide

>60M infested ha
Yield losses often >30%
(*Ostrinia*, *Spodoptera*)

What is environmental impact

Statistician -
compared to what?

Most studies do not compare to current ag
procedures

Some African farmers put insecticide in whorl

- medicine dropper
- pinch of granules

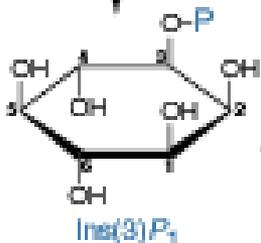
Bt gene being put in maize - but not sorghum

Storage of phosphorus in plants

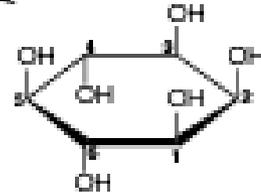
TRENDS in Plant Science Vol.6 No.10 October 2001

Glucose 6-phosphate

myo-Inositol(3)*P*₁ synthase



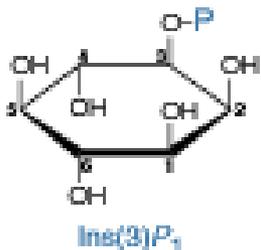
Ins(3)*P*₁ mono-phosphatase



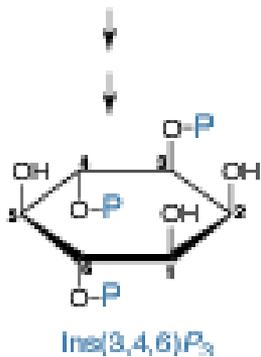
Other pathways that use *myo*-inositol

Early or substrate supply pathways

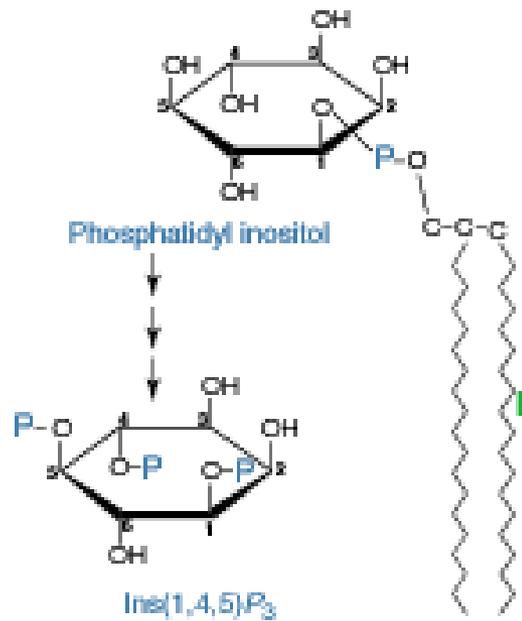
myo-Inositol 3-kinase



Inositol phosphate intermediate pathway



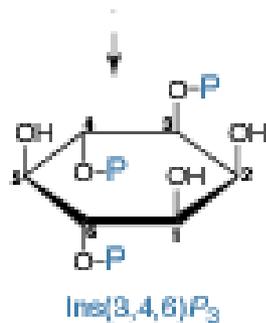
Phosphatidyl inositol synthase



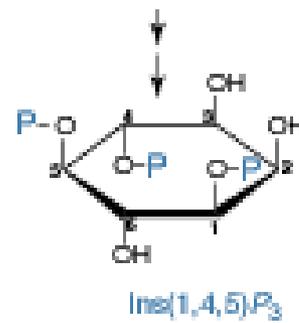
Phosphatidyl inositol phosphate intermediate pathway

Victor Raboy

Inositol phosphate intermediate pathway

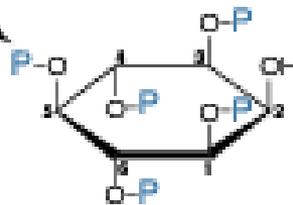


Phosphatidyl inositol phosphate intermediate pathway



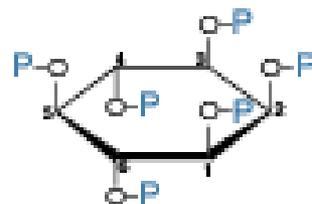
Late inositol phosphate pathway

Inositol polyphosphate 5,6-kinase



Inositol(1,3,4,5,6)P₅

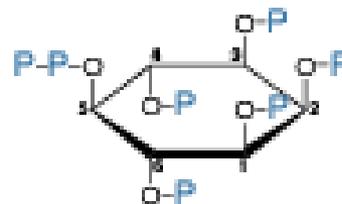
Inositol(1,3,4,5,6)P₅ 2-kinase



Inositol(1,2,3,4,5,6)P₆ or phytic acid

these P groups bind Fe and Zn

Inositol P₆ kinase



5-Diphospho-Inositol(1,2,3,4,6)P₅ or Inositol P₇

Pyrophosphate-containing inositol phosphate

Victor Raboy

Major crops produce ca. 50 M tons phytate/yr
-binding 9 M tons phosphorus - not available to
mono-gastric animals

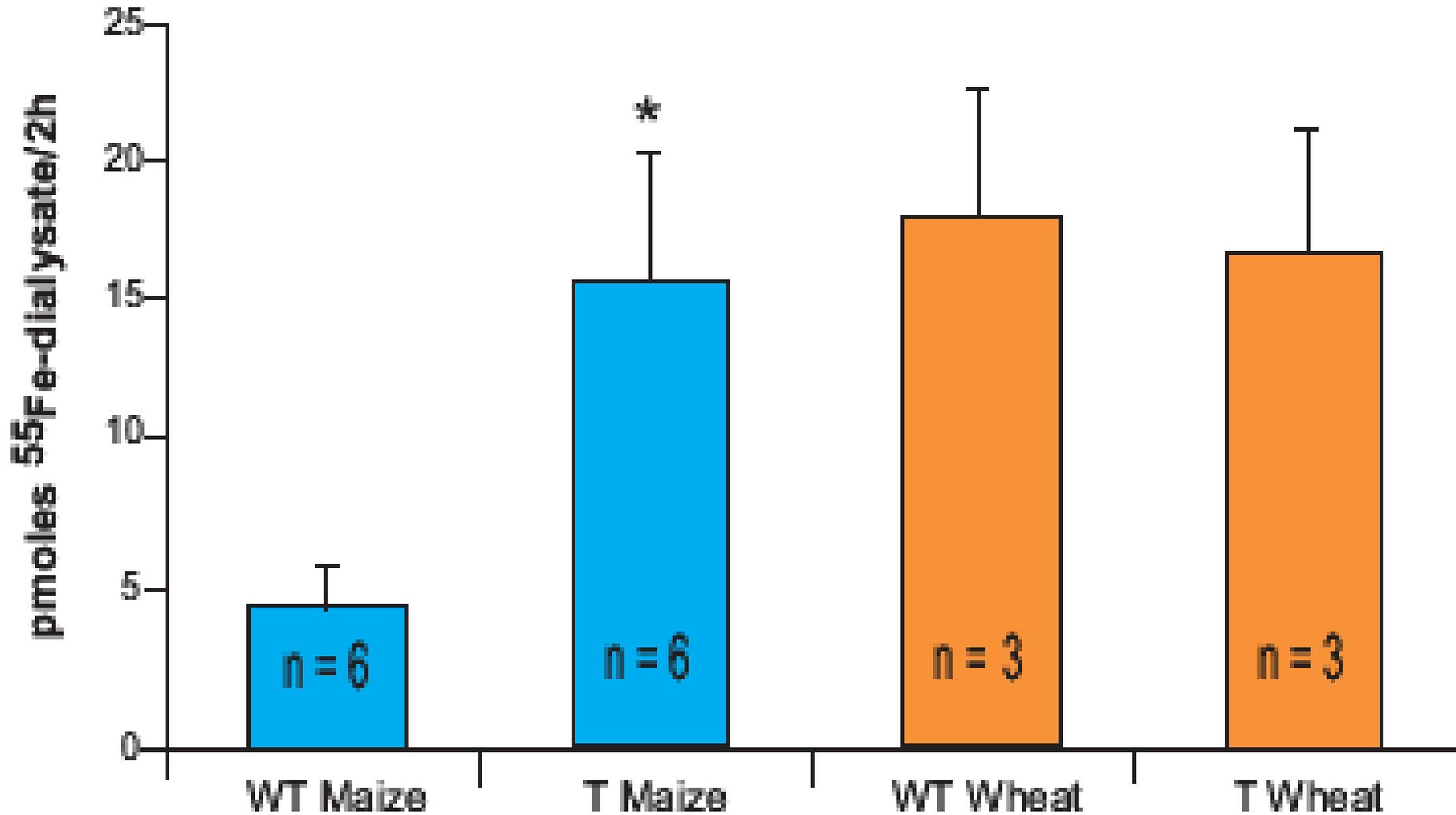
Must add inorganic P to feeds of mono-gastric
mammals, poultry and fish (not ruminates) -
excrete the phytate, which releases pollutant
levels of P

People zinc and iron deficient

Three approaches:

1. add thermostable phytase to feed
(transgenically over-produced by microbes)
2. Lower phytate production by breeding or
transgenics
3. Transform crops with phytase genes

Iron availability in plants with transgenic phytase



Oilseed rape is favored for temperate climates

Is it nice to the environment?

Worldwide, oilseed rape emits ca. 9000 Tons

Methyl Bromide*

Before the ban Europe consumed 18,000T of methyl bromide

Is "natural" MeBr ok and synthetic bad?

Is it ok to double the area - for biofuel?

*Gan, J., et al. (1998) Production of methyl bromide by terrestrial higher plants. *Geophysical Research Letters* 25, 3595-3598

Brassica has a bifunctional methyltransferase

methylates halides to methyl halides (MeBr)

methylates bisulfides to methanethiol

(goes to H_2SO_4 to acid rain)

TDNA disruptive insertion in related

Arabidopsis *HOL* (*harmless to ozone layer*)

gene reduced MeBr >99%

To meet intent of methyl bromide ban and reduce acid rain, must cultivate only transgenic oilseed rape with this gene suppressed; non-transgenic should be banned

Jatropha for biodiesel



www.jatrophaworld.org/

30% oil - seeds get US\$140/ton (optimistic)

- fruits hand harvested
- fruits dried in the shade
- seeds removed by hand

Is *Jatropha* a gimmick to keep the poor poor?



Processing reminiscent of backyard steel mills in China during the cultural revolution

Is “non-toxic”-Mexican *Jatropha* not toxic?

	curcin ^a	phorbol esters ^b	trypsin inhibitor ^c	phytate ^d	saponins ^e
3 <i>Jatropha</i> varieties (average)	102	2.39	20.3	8.9	2.2
“non-toxic”-Mexican <i>Jatropha</i>	51	0.11	26.5	8.9	3.4
soybeans (control)	<0.5	-	3.9	1.5	4.7

^ameasured as lectin haemagglutination; ^b mg/g kernal; ^c mg/g meal; ^d % in dry matter meal; ^e measured as % diosgenin equivalents in the meal. Source: Modified from Makkar et al. ¹⁸

18. Makkar, H.P.S., Aderibigbe, A.O. and Becker, K. (1998) Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. Food Chemistry 62, 207-215.

Websites claim “curcin is heat degradable”
 Quoted citation says “degradable by prolonged autoclaving”

What to do with toxic byproducts?

Websites suggest - Use residue as manure
no environmental impact studies

Could one release a transgenic crop with such
components? *Jatrofraud!* ?

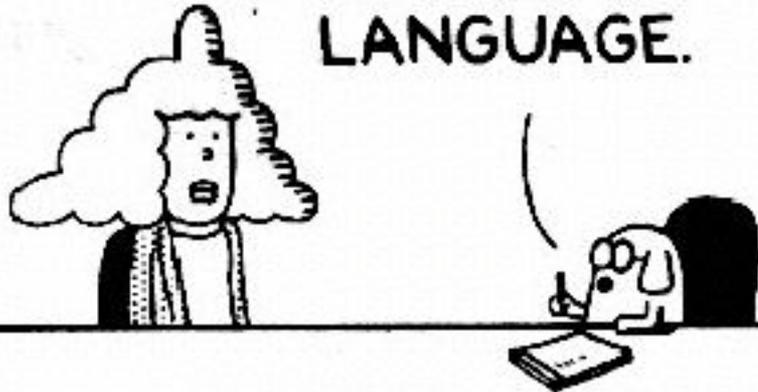
Remember - with soybeans there is more
value from meal than oil....

Where are the economics of discarding
"castropha" meal?

Hype for toxic oilseeds

DOGBERT, THE VP OF
MARKETING

DESCRIBE YOUR
PRODUCT IN TECHNICAL
TERMS AND I'LL TURN IT
INTO MARKETING
LANGUAGE.



ALL THE
PARTS ARE
KNOWN
CARCINOGENS.

"MAKES YOU
APPRECIATE
LIFE!"



Are countries cultivating guinea pigs?
Where is precautionary approach?

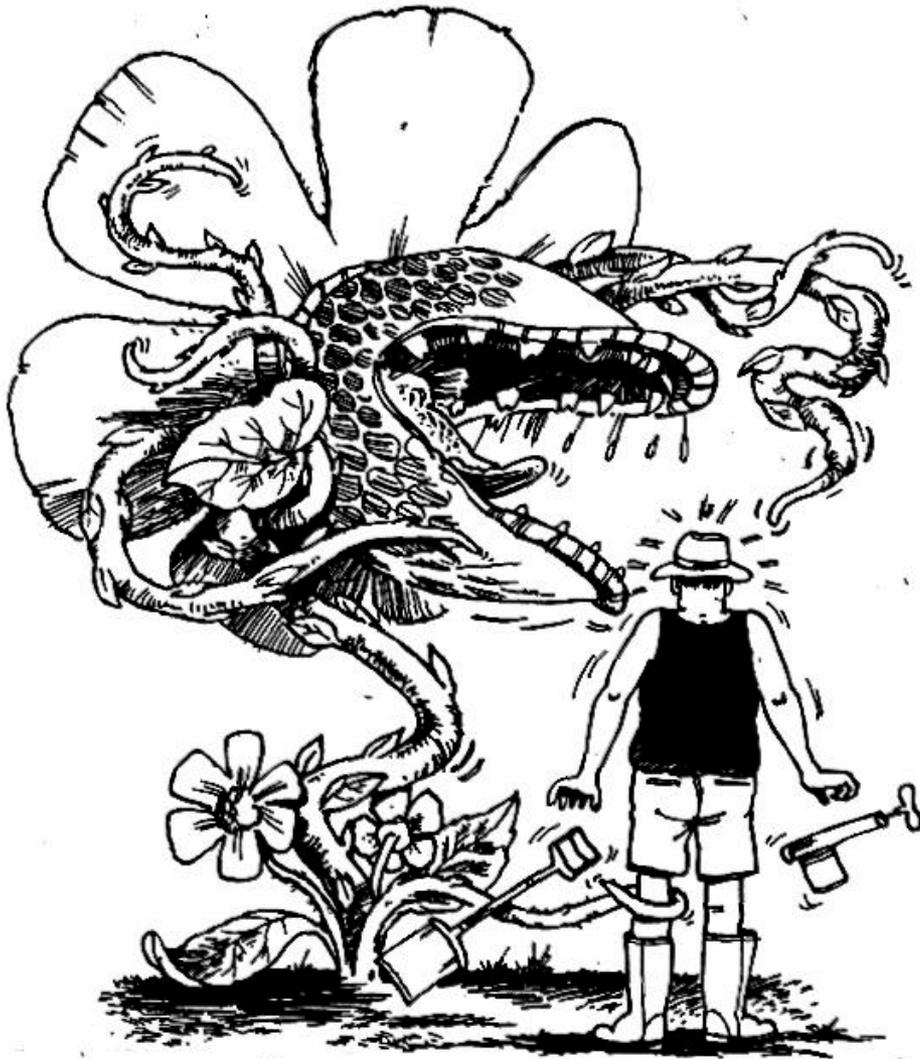
If you want "Castropha" as an oil crop -
engineer or breed:

- dwarfing (increase harvest index)
- single stalk (high IAA?)
- Antishattering - fruits dry on stems
 - machine harvesting and threshing
- RNAi curcin genes
- RNAi terpene synthase to rid of phorbols
- better yield, oil content / quality

The engineered crop might then be
environmentally/human safe to grow

The press warns
about

SUPERWEEDS



Beat the winter chills with **Trust**

**Giant buttercups in
nightmare invasion**

The implications are that natural ecosystems are endangered



"Frankly, I think we'll regret introducing these organisms into the environment."

Peter Raven dispelled this

The newspapers tell us:

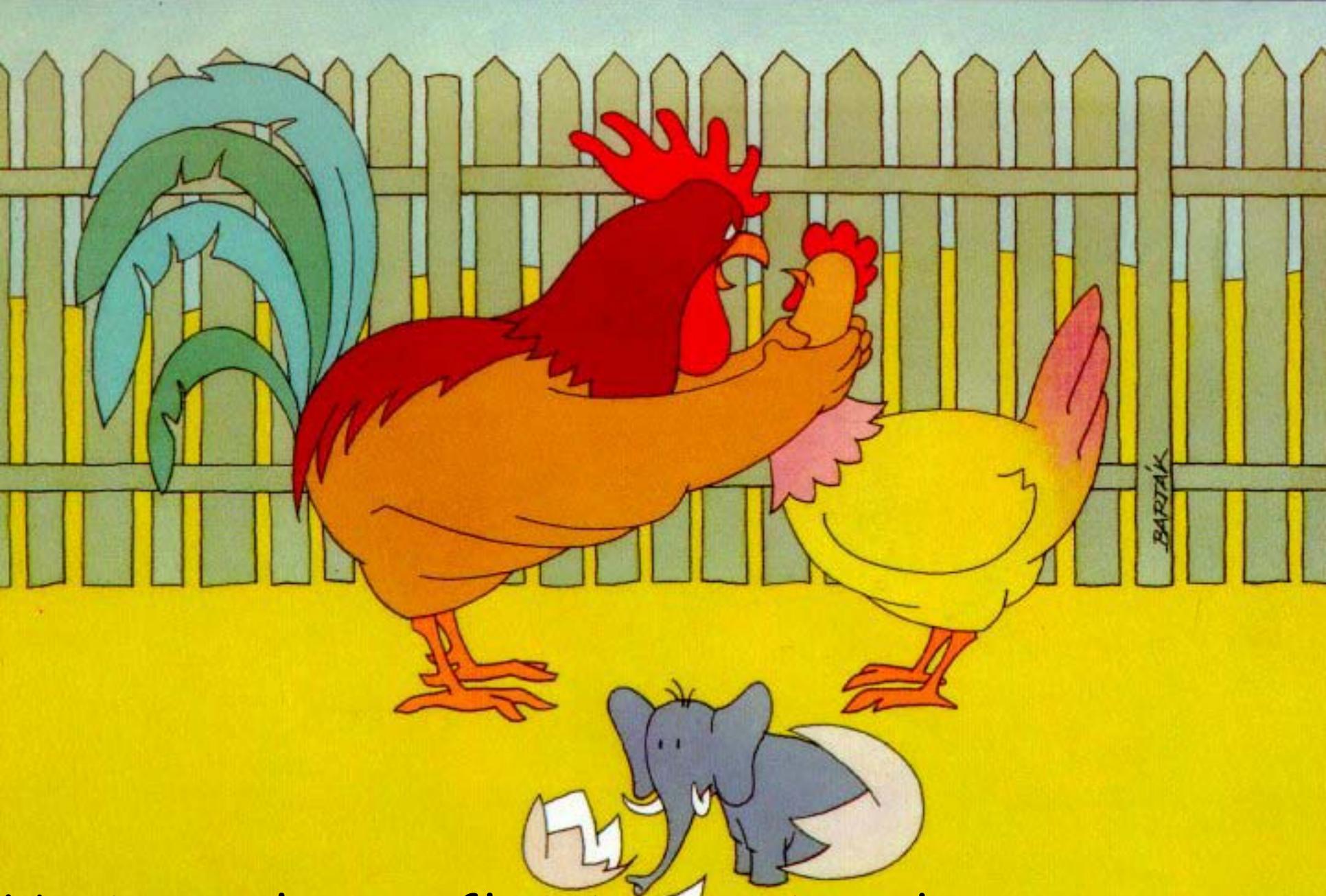
A THOUGHT FOR THIS WEEK

[From the Phoenix (Ariz.)

Arizona Republic]

RIVERSIDE, Calif.—The greatest danger posed by genetic engineering of plants may come from sex with weeds.

Horizontal gene flow often implied



Horizontal gene flow is rare in eukaryotes

Dangerous Liaisons?

When Cultivated Plants
Mate with Their
Wild Relatives



Norman C. Ellstrand

Good source of
information

Does not
differentiate
between wild and
weedy

Usually the problem
is weedy

Two perceived problems from transgenic crops

1. Can the crop become a volunteer weed?
2. Can the crop introgress transgene trait into related wild species?

These problems are not unique to transgenic crops; they are general to all crops.

The question usually asked is:

Can transgenic trait introgress (cross into and become established) in related wild species?

If the answer is "yes" *Introgression Fever* sets in.

If the answer is yes,
the question "*so what?*"
should be asked

"So what?" means:

How likely is introgression? when? where?

What are the consequences?

Can the related hybrid or its offspring become weeds?

Does gene have selective advantage?

Will there be over-riding benefits till introgression occurs?

Some crops have severe weed problems from related weeds:

Rice

Sorghum

Wheat (limited areas - diagonal only)

Oilseed rape

Sunflowers

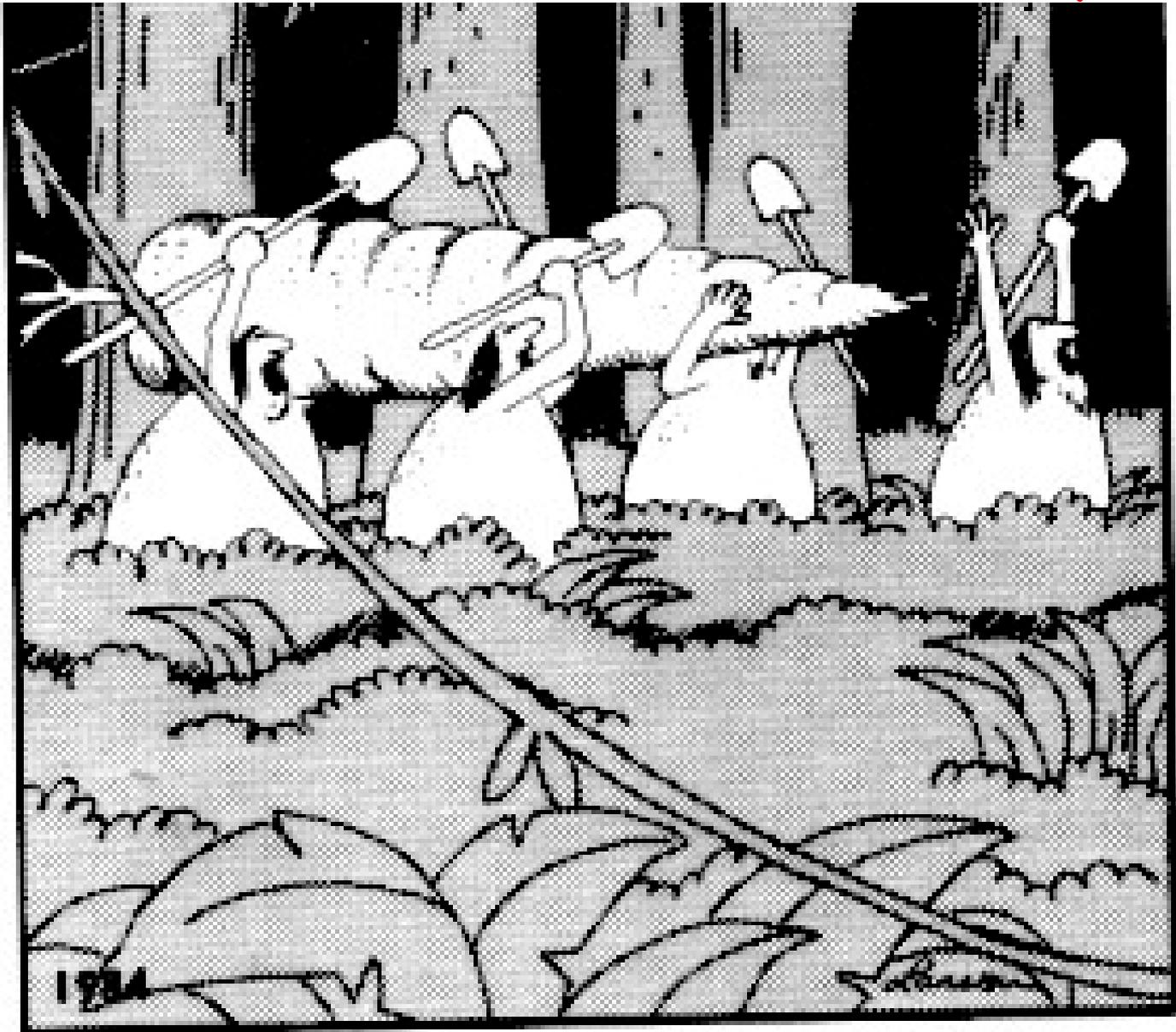
Sugarbeet

Carrots

No problems in corn, soybean or potatoes

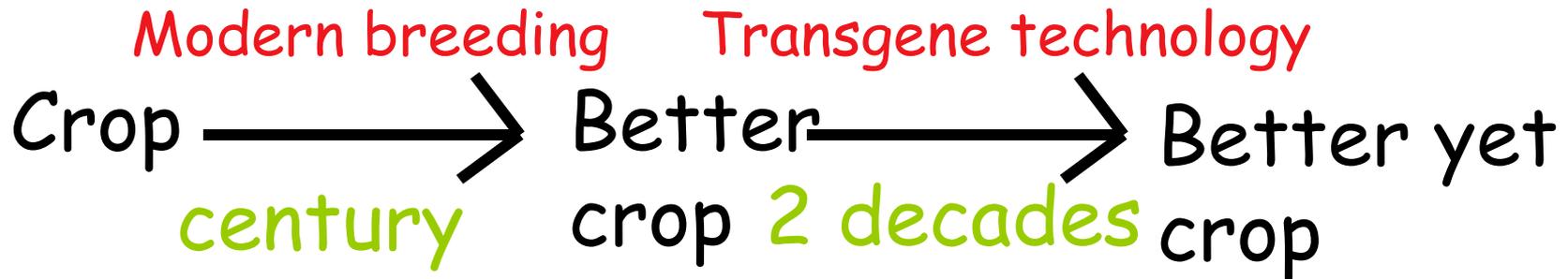
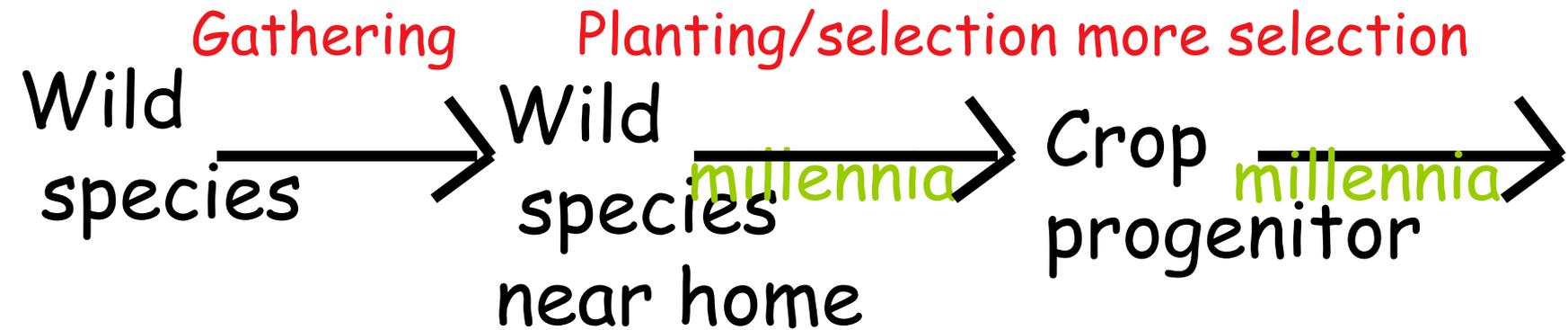
How did these weed problems evolve?

Ancient man has domesticated many crops



Early vegetarians returning from the kill

Stages of Domestication



At the same time:



Wild Species $\xrightarrow[\text{centuries/millennia (recessive traits)}]{\text{Domestication}}$ Crop

Crop $\xrightarrow[\text{annually}]{\text{Shattering}}$ Volunteer weed $\xrightarrow[\text{shattering secondary dormancy height, branching, etc.}]{\text{cumulative (dominant traits) De-domestication}}$ Feral weed

Ferality is thus an evolutionary problem



"I still say it's only a theory."



Move from transplanting to direct seeding

Transplanted field
- ca. 10% yield loss predicted

Feral ⇒

A close-up photograph of a rice field. The plants are mostly green, but many have yellowed, indicating stress or maturity. The text 'Feral ⇒' is overlaid on the image, pointing to a specific plant that appears to be a weed or feral rice variety. The background is filled with similar rice plants, some showing signs of being overgrown or weedy.

**Taller than crop and matures earlier
- shatters**





Found in one Malaysian paddy



cultivated

A



B



C



D



E



F



G



H

Another case: *Sorghum bicolor*



Feral/weedy type
(shattercane)



Mature Panicle of Shattercane

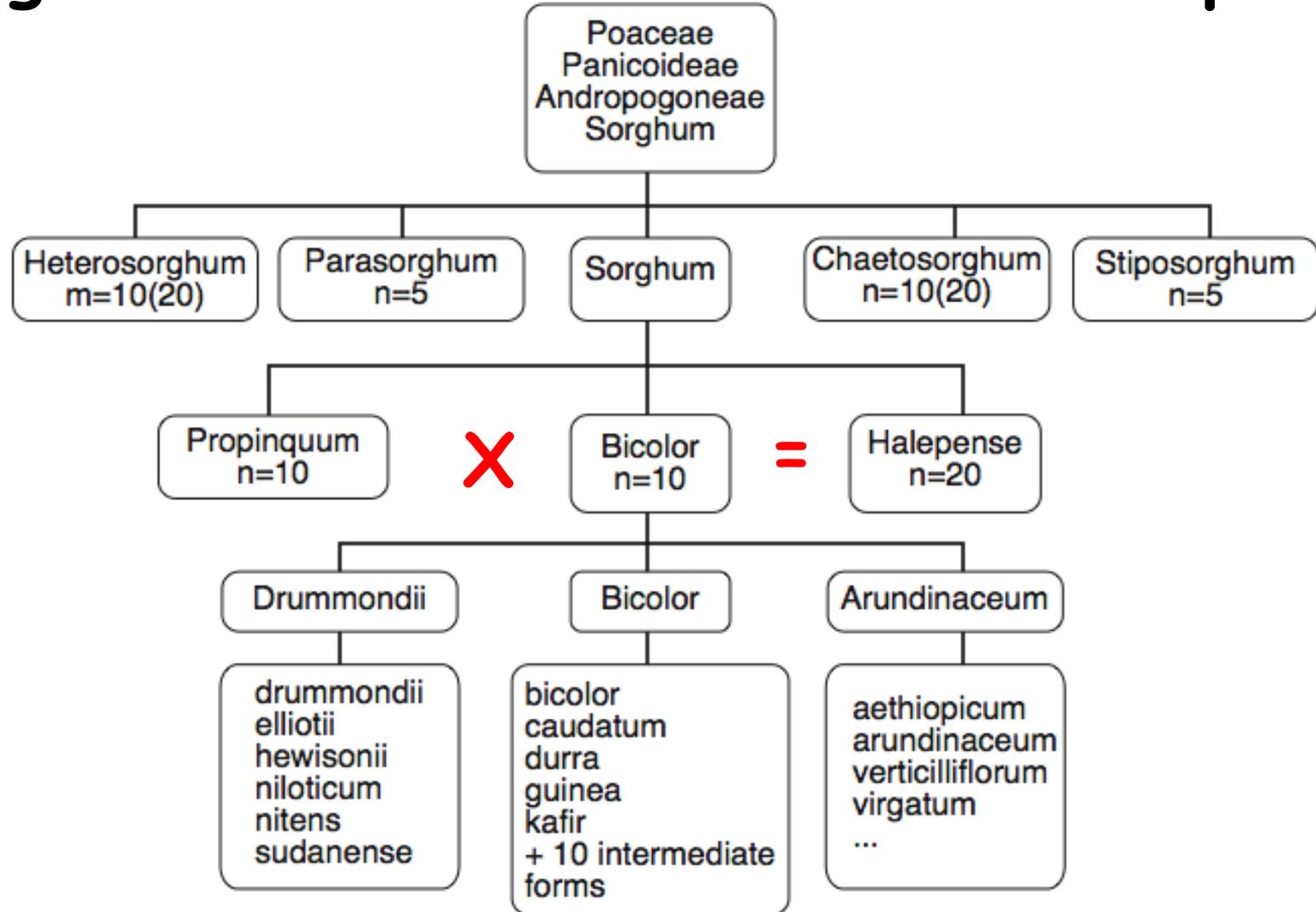


Shattercane Plants in a Corn Trial



Few selective herbicides can control feral/weedy sorghum in sorghum or maize

Sorghum crosses with a native wild species



Sorghum halepense

johnsongrass

Stupidly
spread by
man



Photo by:
Richard Old
www.xidservices.com



Evolved resistance to
ALS, ACCase and
glyphosate

**WE HAVE MET
THE ENEMY
AND HE IS US.**



We must learn about the feral weedy enemy, and make sure that the right things get done to keep it under our control!

Crop Fertility *and* Volunteerism

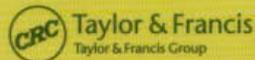
Edited by
Jonathan Gressel



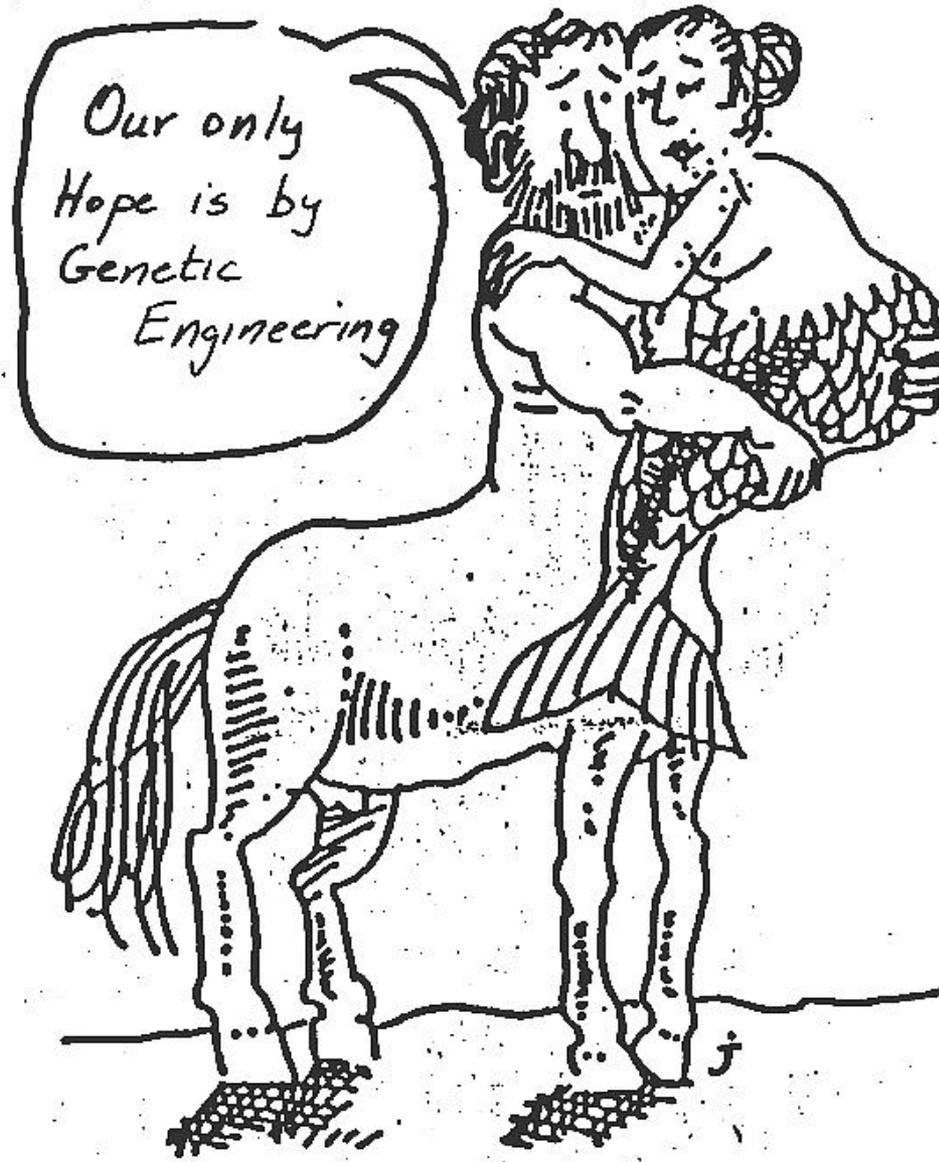
Fertility
→



ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT



For more information
- see the book



Generate transgenic, herbicide resistant crops

Look at Imi-R (Clearfield) rice - do not repeat mistakes

A non-transgenic - herbicide resistant rice
Gave excellent control of weedy rice
for 2-3 years

Resistant gene introgressed into weedy rice
technology lost

Must prevent gene flow
- by not making transgenic rice/sorghum?

At places where
stupidities flourish

Wisdom is considered a
weed

Can wisdom be used to delay / preclude
introggression?

Two types of transgenic strategies proposed:

- Containment

- keeping the gene in the crop

- Mitigation

- preventing establishment and spread of escaped transgenes

How do they work?

Containment Strategies

"Terminator" or similar "GURT" (genetic use restriction technology)

Chemically induced promoters

Insert gene(s) of choice into plastome

Plastids maternally inherited

-no pollen transmission

"Repressible seed-lethal system".

Schernthaler et al *PNAS* 100, 6855-9, 2003

Single generation transformation

Use of attenuated viruses

to transiently transform crops

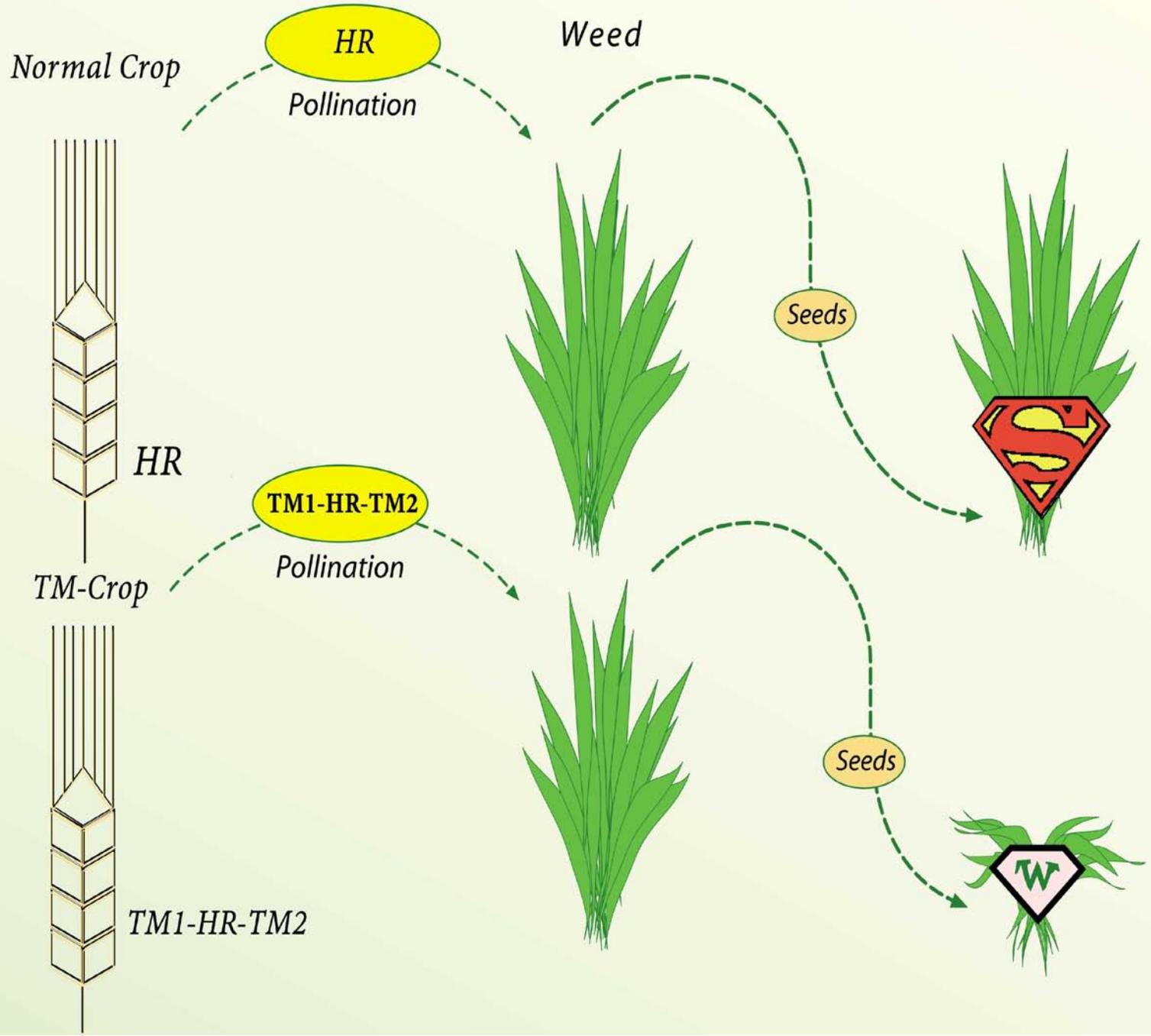
Mitigator technology

A primary (e.g. Bt, herbicide resistance) transgene is coupled in tandem with mitigator genes that are positive or neutral to a crop but deleterious to offspring

Need genes that are carriers of incompetence
in volunteers and offspring, ok in crop



"It isn't only that Fenwick is incompetent, but he seems to be a *carrier* of incompetence."



Two types of mitigators

- Eco/morphological

Unfitness of hybrids/offspring is due to ecological or morphological traits

- Eco/biochemical

Unfitness due to super-susceptibility to rotational herbicides

Both can be transgenically stacked with each other

Lets think about some TM genes by looking at the differences between rice and red rice

Eco-morphological differences

Domestic rice

dwarf / semi-dwarf
ca. 100% harvested
ca. 100% germinates

Feral & wild rices

tall
ca. 80% shatters
secondary dormancy

Genes for dwarfing, anti-shattering or no secondary dormancy would turn a weed into a non-competitive entity

TM genes that would be neutral or useful for the crop but deleterious to the related weed

Dwarfing has been transgenically achieved with:

- antisensing many genes for GA biosynthesis
- introducing truncated GA receptor gene (ΔGAI)
- antisensing brassinosteroid biosynthesis genes
- introducing antisensed phytochrome genes
 - prevents shading response in a crop

(no perception of end of day FR) PHYP

apophytochromes (PHYA, PHYB, PHYE)

PHYB- *Robson et al. Nature Biotech 14: 99, 1992*

PHYD - *Aukerman et al. Plant Cell 9: 1317, 1997*

PHYE - *Devlin et al. Plant Cell 10: 1479, 1998*

Antiweediness Transgenic Mitigation Traits

(Anti) - Seed Shattering

- Prevents weed replenishing seedbank
- Prevents crop from being volunteer
- Prevents crop loss

Genes:

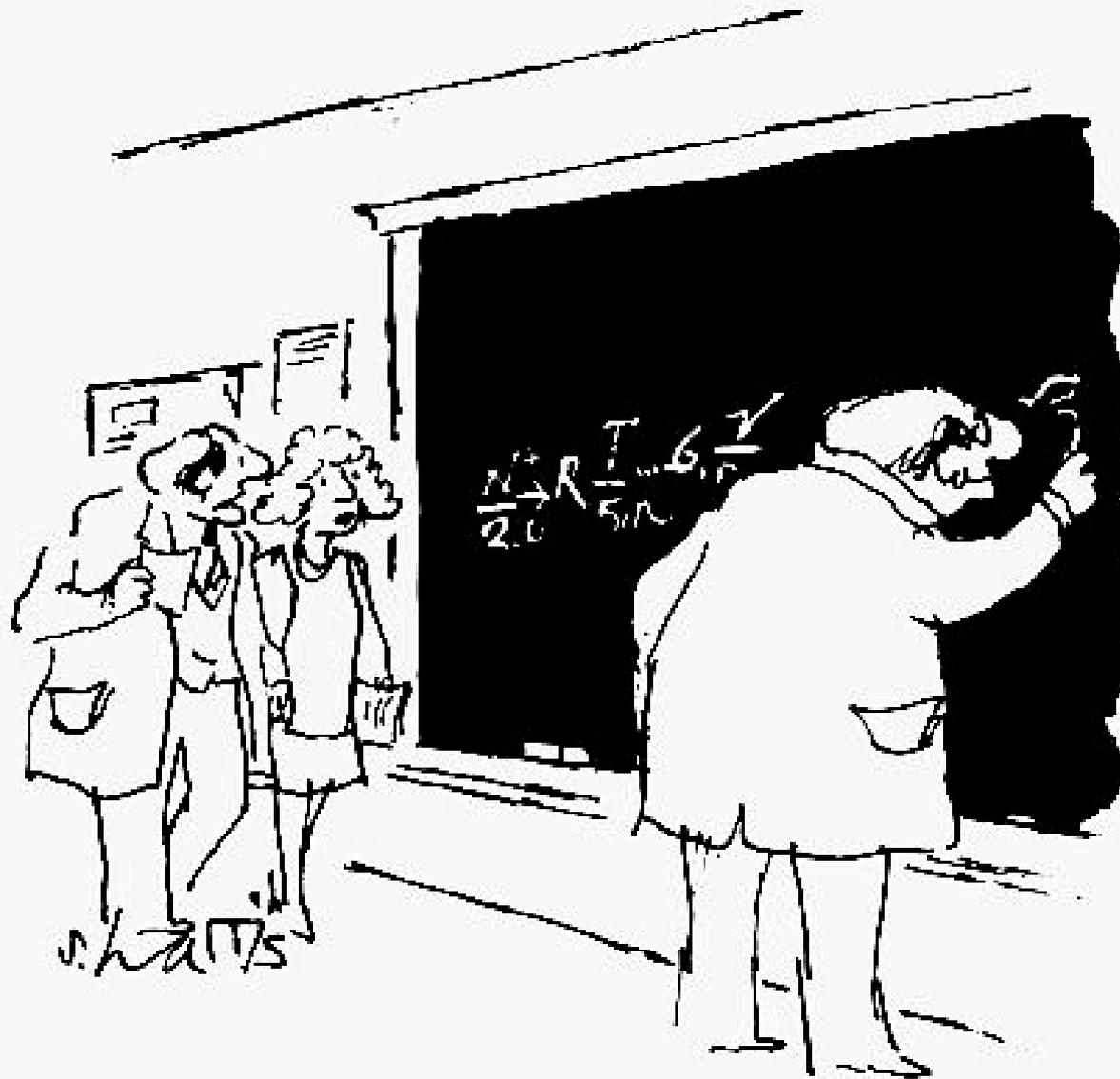
- Determinacy (uniform ripening/harvest)
- "*Shatterproof*" (no dehiscence layer)

Antiweediness Transgenic Mitigation Traits

(Lack) of secondary dormancy

Genes:

- Arabidopsis abscissic acid insensitive
Steber et al. *Genetics* 149: 509, 1998
- enhance gibberellin biosynthesis
- Abolish light requirement (phytochrome)
- (Anti) wall thickening



"I think he's crossed that thin line between science fiction and fantasy."

Will studying a model of Beethoven teach why he was stone deaf?



Any evidence Transgenic Mitigation works?



Hani al-Ahmad exPhD student

Wild Type

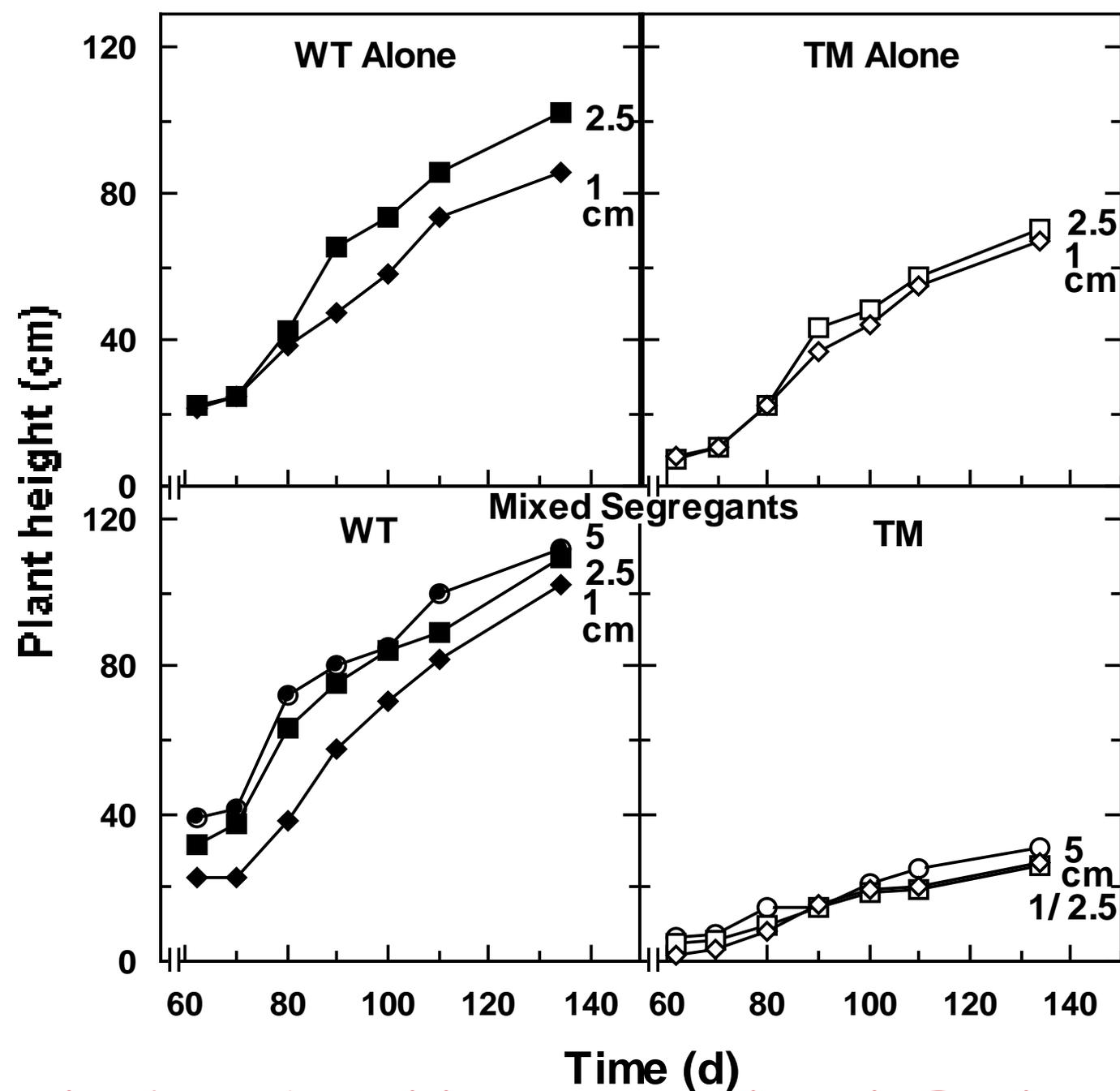


Generated
transgenic
Tobacco



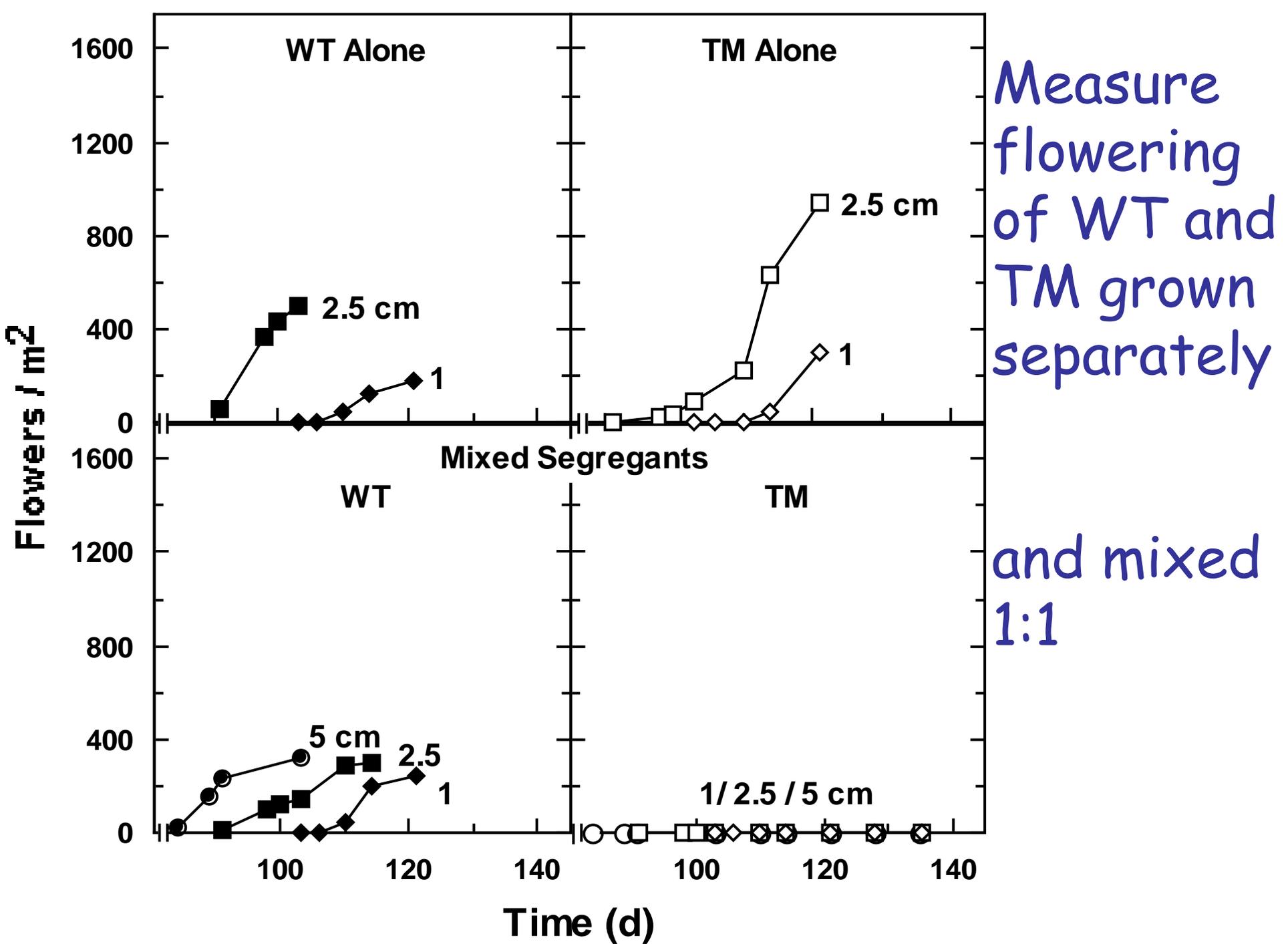
Non-transg. Tobacco
control-4
12 weeks

Tobacco Tr2
12 weeks

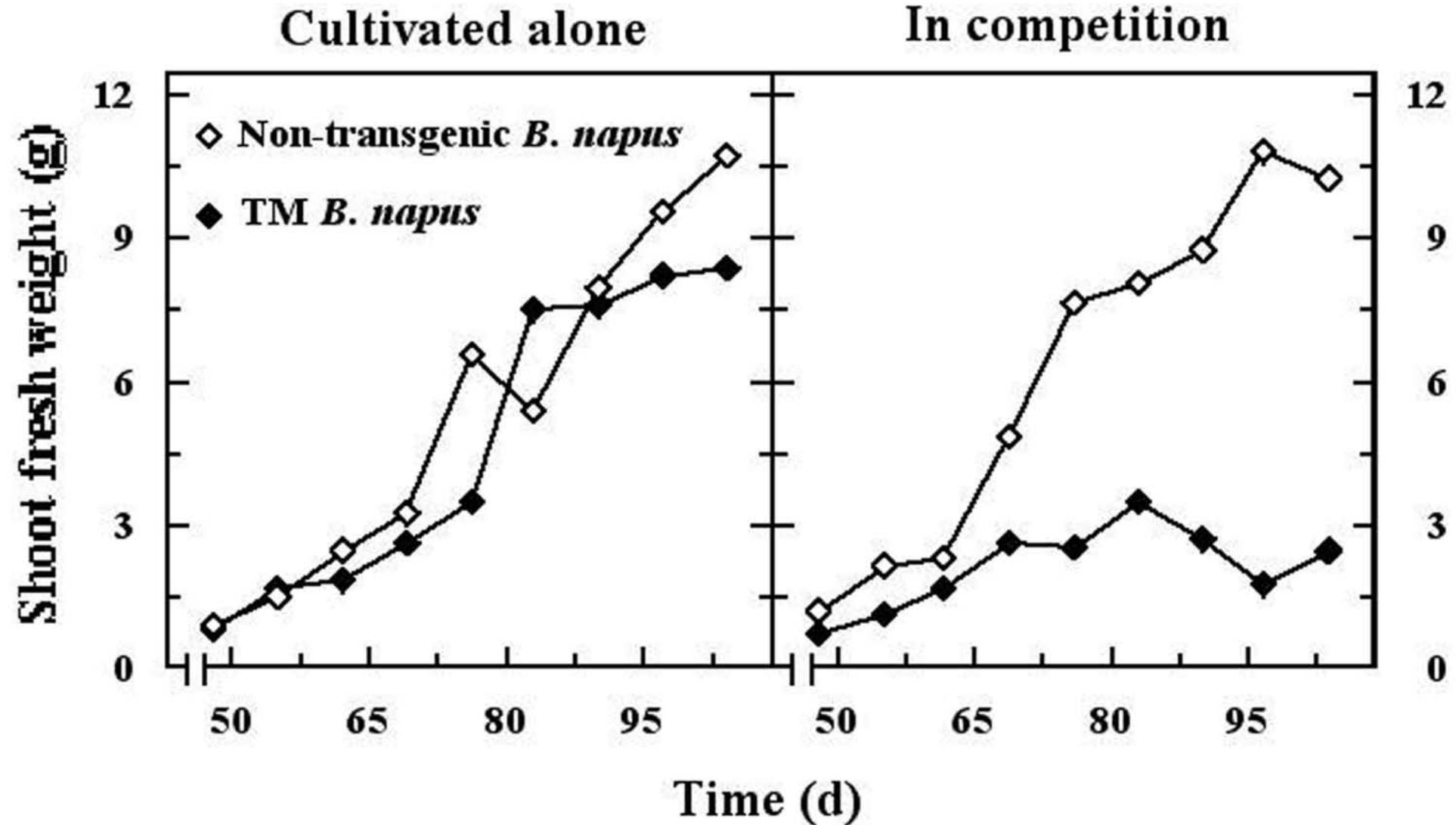


Measure height of WT and TM grown separately

and mixed 1:1



Similar results with dwarf TM oilseed rape



Al-Ahmad et al. Plant Biotech J. 4:23-33, 2006

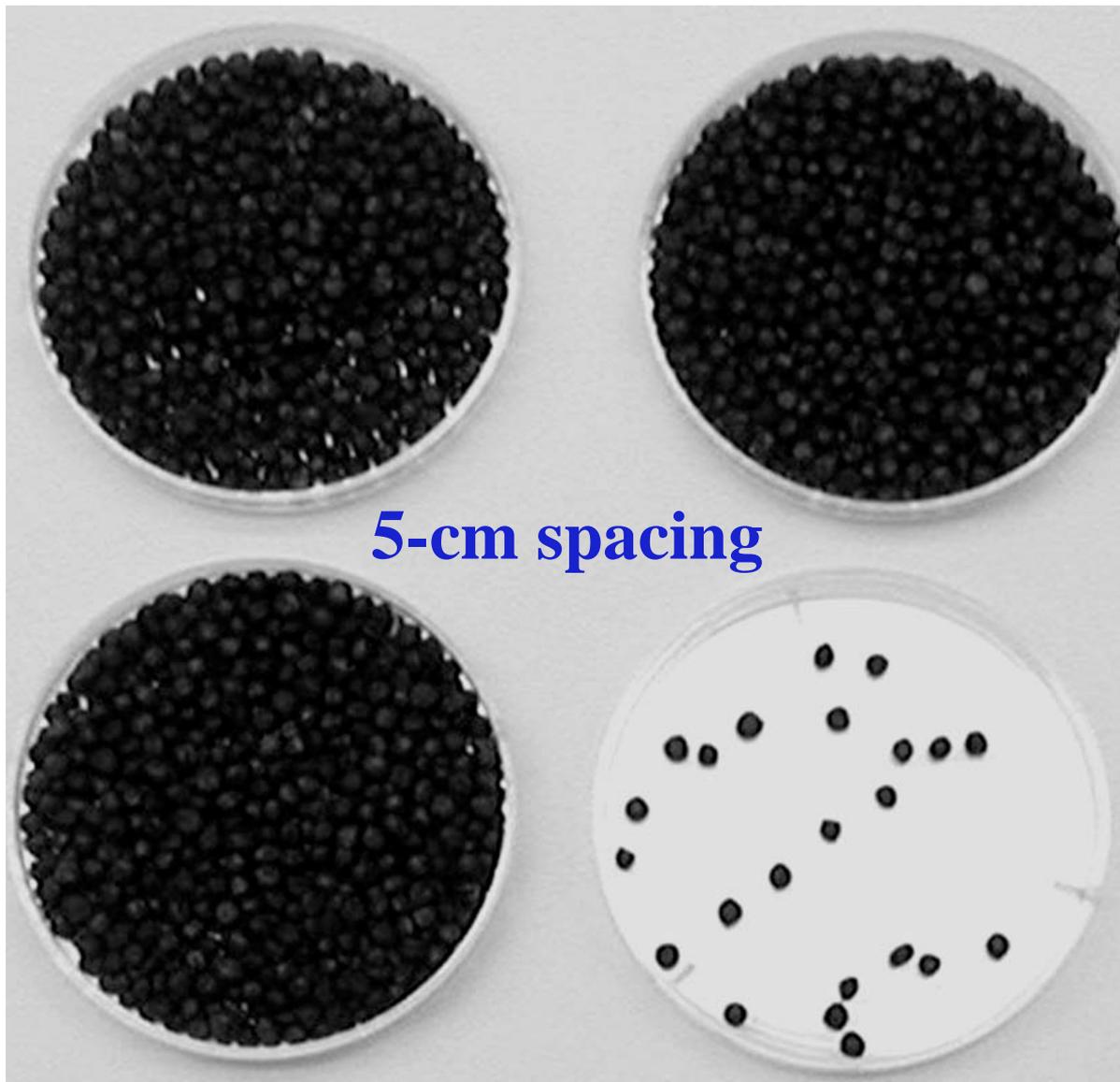
Oilseed rape (*B. napus*)

yield/plant

Non-transgenic

TM

Alone



Mixed (1:1)

Transgenic mitigated oilseed rape has high productivity and low competitive fitness

Biotype	Productivity grown alone (mg/plant)	Reproductive fitness in competition with <i>B. napus</i>
---------	---	---

B. napus NT

320

B. napus TM

503

0.20

B. rapa

119

(Al-Ahmad and Gressel, Plant Biotech J 2006).

- increased yield when grown by itself
- unfit in competition with wild type
- unfit when crossed into *B. rapa*

Thus

Transgenic mitigated *B. napus*:

- increased yield when grown by itself
- unfit in competition with wild type
- unfit when crossed into *B. rapa*

Works in the field -Neal Stewart's group

Similar mitigation strategies to prevent establishment of weedy rice/sorghum -
many useful genes already cloned for
dwarfing and anti-shattering

Eco-biochemical gene flow mitigation

OPEN ACCESS Freely available online



A Built-In Strategy for Containment of Transgenic Plants: Creation of Selectively Terminable Transgenic Rice

Chaoyang Lin¹, Jun Fang¹, Xiaoli Xu¹, Te Zhao¹, Jiaan Cheng¹, Juming Tu², Gongyin Ye¹, Zhicheng Shen^{1*}

¹ Institute of Insect Sciences and State Key Laboratory of Rice Biology, Zhejiang University, Hangzhou, China, ² Institute of Crop Sciences, Zhejiang University, Hangzhou, China

PloS One 3(3):e1818.

Doi:10.1371/journal.pone.0001818

(Despite title not containment)

1. Use cv A and glyphosate
2. Use cv B and glufosinate
volunteers and hybrids are super-sensitive to glufosinate - killed
3. Use cv A and glyphosate
volunteers and hybrids super-sensitive to glyphosate - dead
4. Use cv B and glufosinate
volunteers and hybrids super-sensitive to glufosinate - deceased
5. Use cv A and glyphosate
volunteers and hybrids killed by glyphosate
6. Use bentazon + selective graminicide
bentazon kills super-sensitive volunteers /hybrids

Such a rotation would:

- kill weedy rice 5 of 6 seasons
- prevent establishment of hybrids with weedy rice - all seasons
- kill herbicide resistant *Echinochloa* 5 of 6 seasons (or 6 of 6)
- extremely delay evolution of all weed resistances to all herbicides used

Will work even better if eco-biochemical mitigation coupled with morphological mitigation

In summary - learn from:

- Non-transgenic herbicide resistance rapidly appears in weedy rice - **Cibus Genetics**
- Transgenic herbicide resistance could allow selective control of weedy rice / other weeds - but genes could flow
- Two types of strategy to deal with gene flow:
 1. Containment
 - probably always leaky - escapes can propagate
 2. Mitigation
 - introgressed progeny always less fit - stay at low frequency unless selection pressure

- Transgene flow can be mitigated
- Evolution of herbicide resistance delayed

Best strategy:

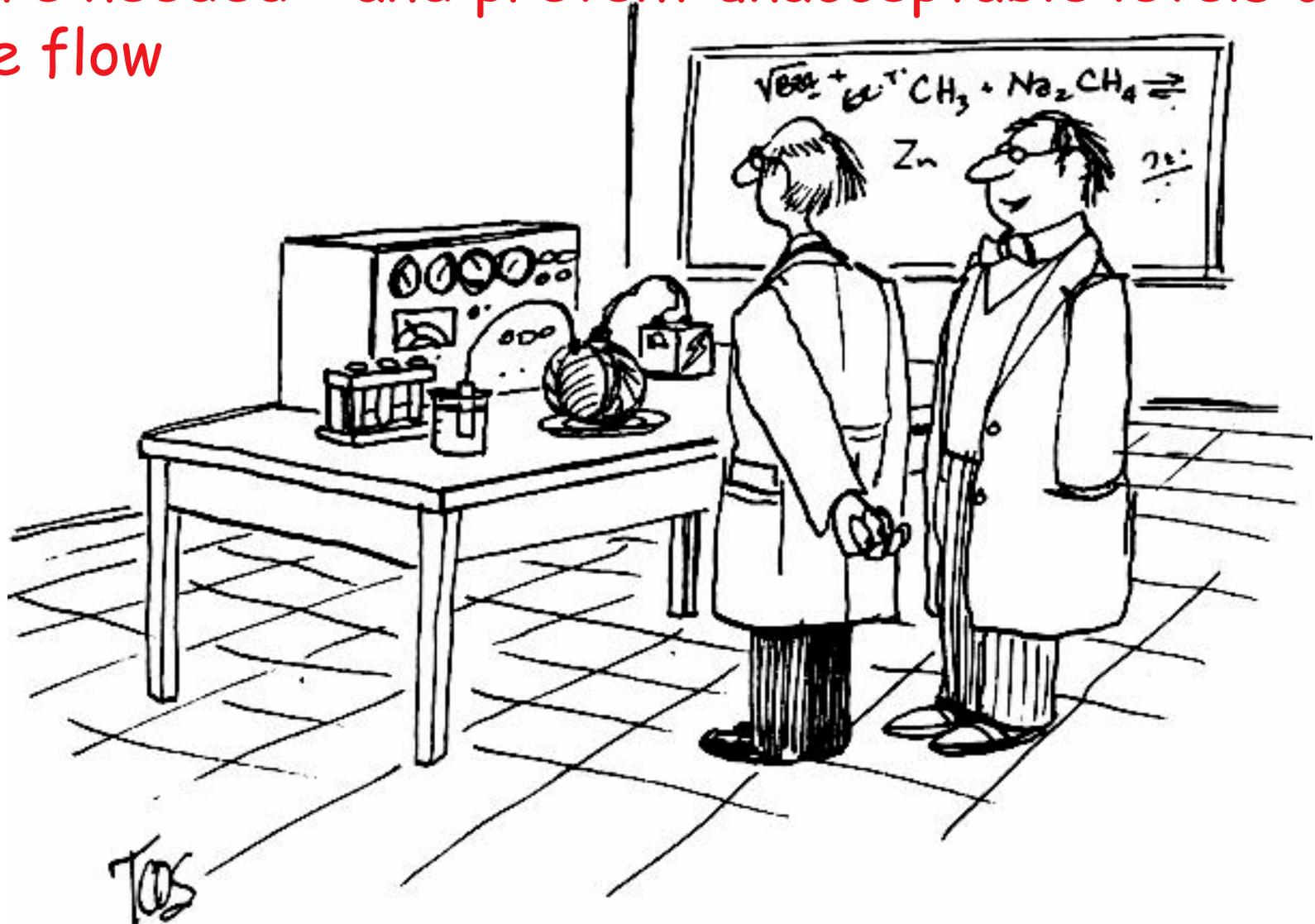
Stack containment with mitigation
get unfit escapes

There are severe regulatory problems to be overcome - multigene constructs

Industry must collaborate to compete with weeds

Otherwise herbicides lost!

Biotechnology can prevent / mitigate transgene introgression - regulators should ensure it is done where needed - and prevent unacceptable levels of gene flow



TOS

"The cabbage is completely under our power."

Only with hand me down genes



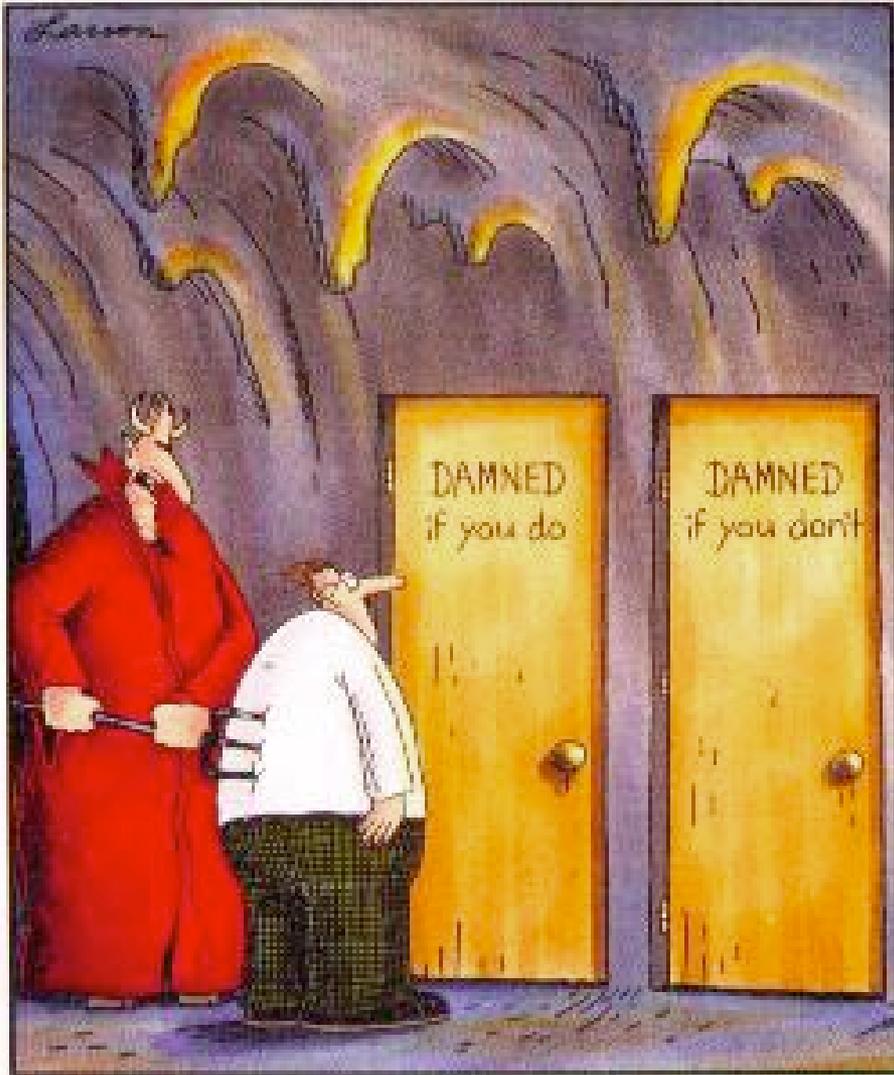
Hand me down genes:

Bt

Glyphosate resistance



The developing world needs new genes



We must decide:
Use biotech to help
or
Sit and do nothing

"C'mon, c'mon—it's either one or the other."

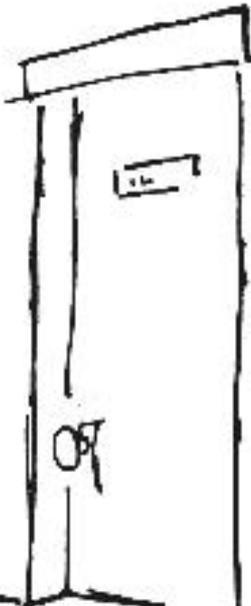
The world needs solutions

- must consider transgenics to supply them

We in the "west" need more modesty
We should beware of cultural imperialism



RESEARCH INSTITUTE



UNANSWERED
QUESTIONS
→

←
UNQUESTIONED
ANSWERS

J. Morris

