Domesticated biofuel crops as a solution for resource limitations

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כפר הנופש רמות

Outline

Zero Generation Biofuels
The problems – food vs. fuel
First Generation Biofuels
Why genetic Engineering
Second Generation Biofuels
- Lignocellulosics for ethanol
- Oils for biodiesel

Summary

Traditional biofuels

← India

Africa

↓

Inefficient
Polluting
Environmentally
negative

Can we do better?

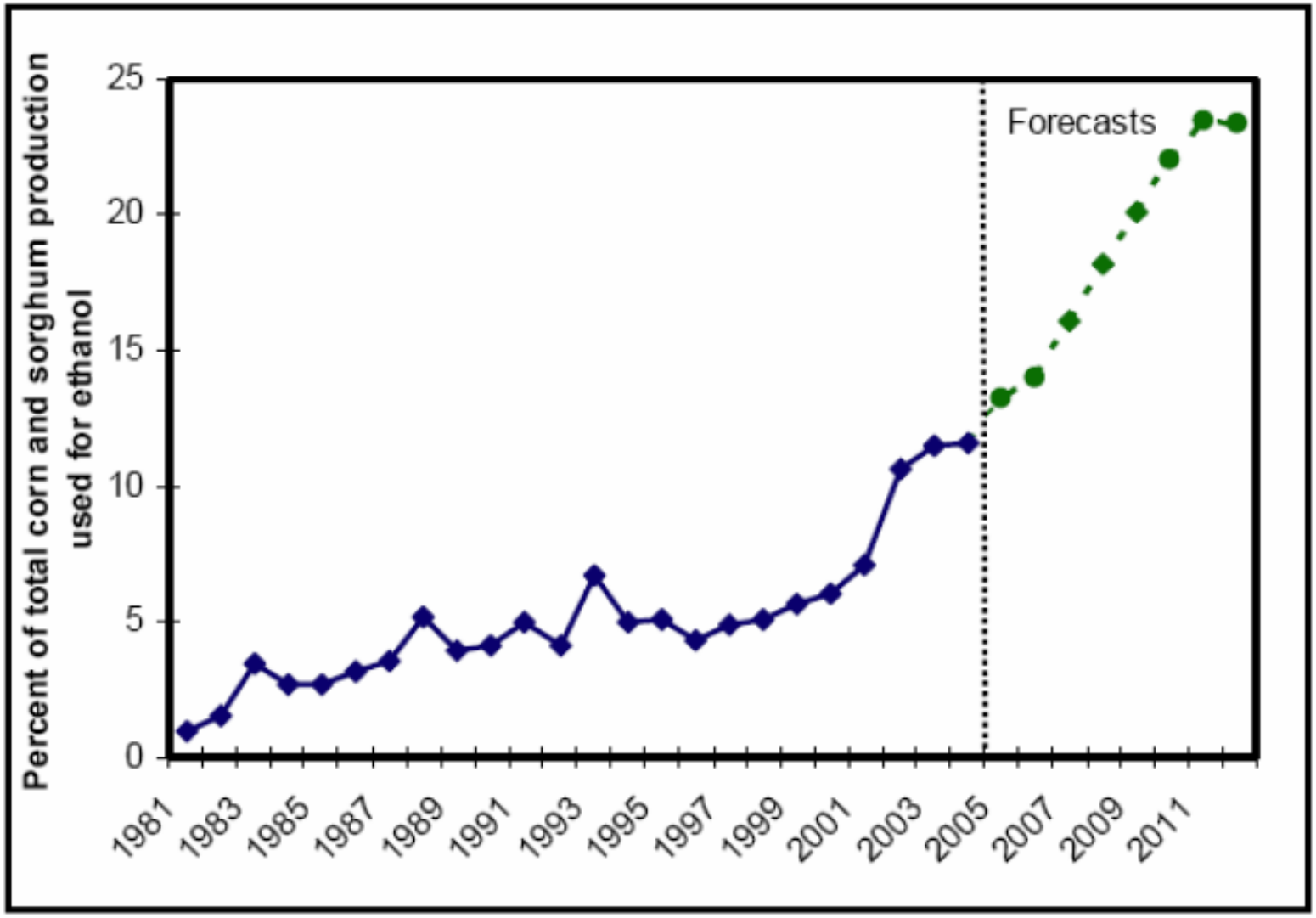
In temperate areas the traditional biofuel was oats:

Cultivated on ca. 20% of land

Oats fueled all of farming

Fueled. mules, horses and laborers

Ethanol: an increasing portion of the US maize crop



Source: Kansas State University

Figure 7. Percent of corn and grain sorghum production used for ethanol production, 1981-2012 Taken from report by Dhuyvetter et al., and published by Agricultural Issues Center, Univ California, Nov. 2005

What are world implications?

Biofuels: Good News/Bad News to developing world

Bad news: no more cheap/free grain for food security in time of famine

Good news: US not "dumping" subsidized grain, sold below production costs

Developing world farmers can now compete

easily triple yields

Biofuels: Good News/Bad News to developed world farmers

Good news to grain farmers - prices stable

Bad news to dairy/beef/chicken/hog farmers - grain prices high…

Bad news to consumers - do not lower fuel prices, higher food costs

Deutsche Welle
Energy | 23.04.2007

Germany's Cheap Beer Tradition Under Threat From Biofuels

The popularity of biofuels is affecting the price of Germany's most cherished beverage

Germans will have to dig deeper in their pockets to enjoy their beloved beer in the next few months as barley is increasingly displaced in the country's fields by heavily subsidized crops used for biofuels.

Ethical question Europe must ask:

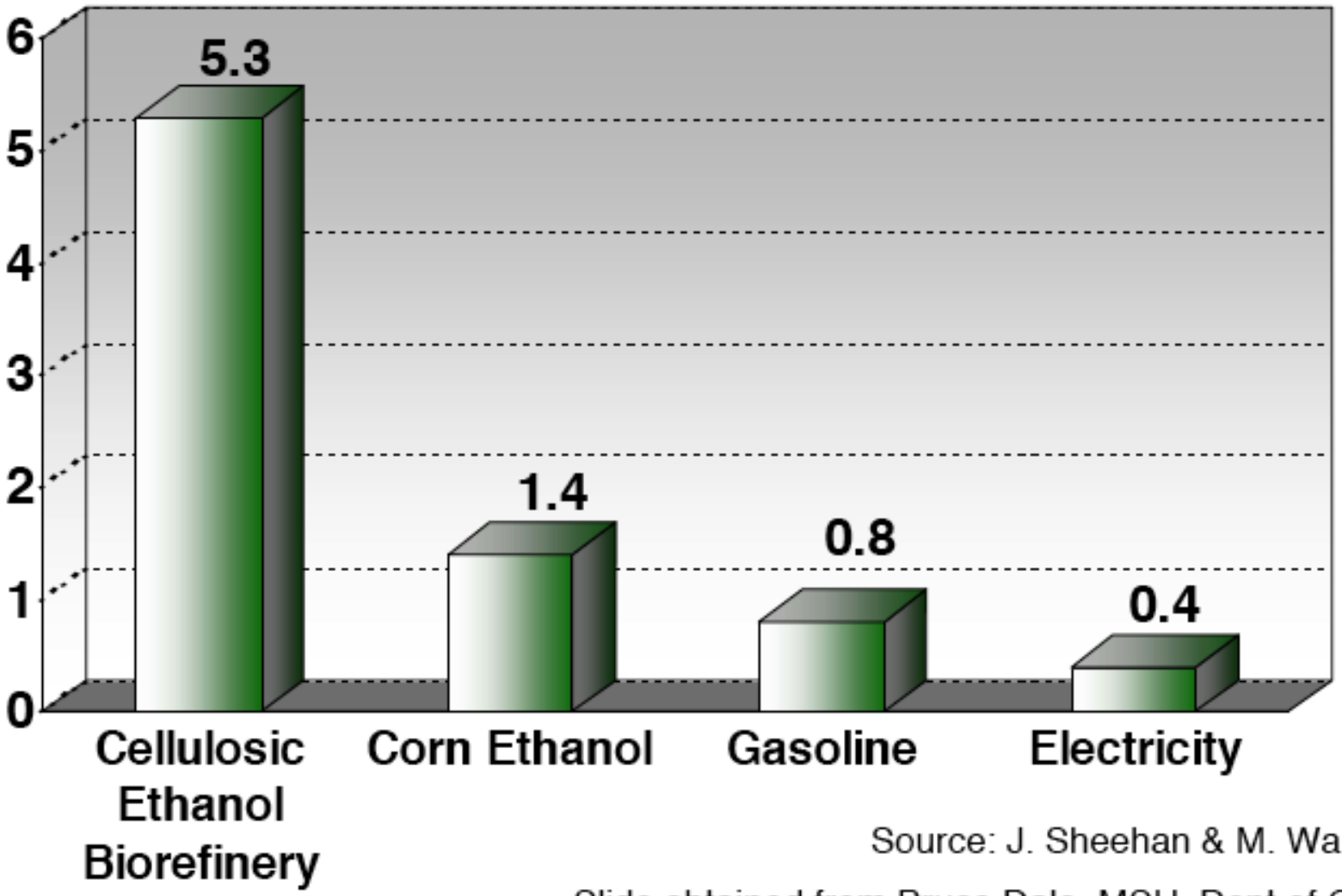
Is it right to support eliminating rainforest and jungles elsewhere for Europe to import soy/palm oil for biodiesel so that Europe can preserve "Landscapes"?

Ethical question all must ask:

Is it ethical to drive a big car on biofuel considering the effect on food prices and availability?

Net gain is low from maize grain ethanol

$$\text{Fossil Energy Ratio (FER)} = \frac{\text{Energy Delivered to Customer}}{\text{Fossil Energy Used}}$$



Source: J. Sheehan & M. Wang (2003)

Slide obtained from Bruce Dale, MSU, Dept of Chem Eng

But this depends on how calculated

Most calculations do not include:

- accounting of byproducts
- recent advances
- appropriate "systems boundaries"

Dale (Biofuels Bioprod. Bioref . 1:14-17, 2007) claims such calculations inappropriate - should be based on ability to replace petroleum or on greenhouse gasses produced per km driven

Domestication

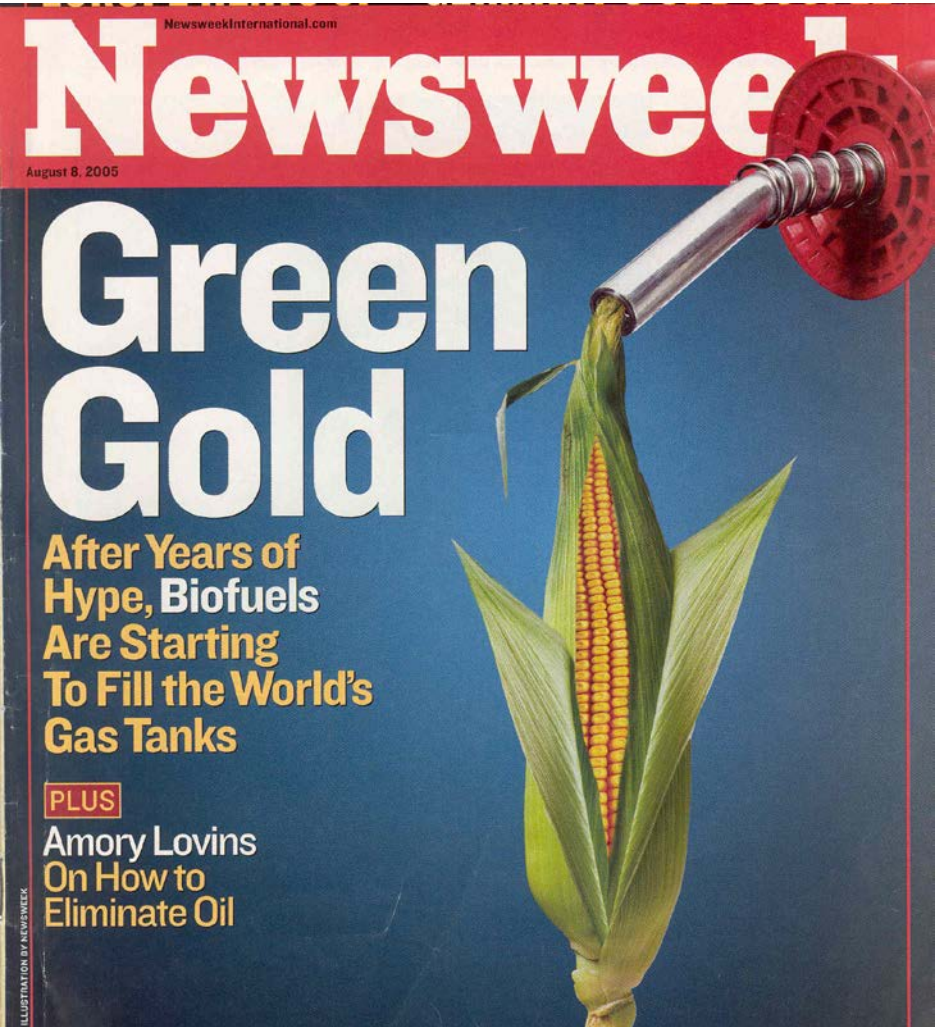
Hybrid corn

Corn ca. 1492

Teosinte- the progenitor

"New" opportunities:

Where will we get food and fuel with the available land?



Biofuel possibilities:

2nd generation

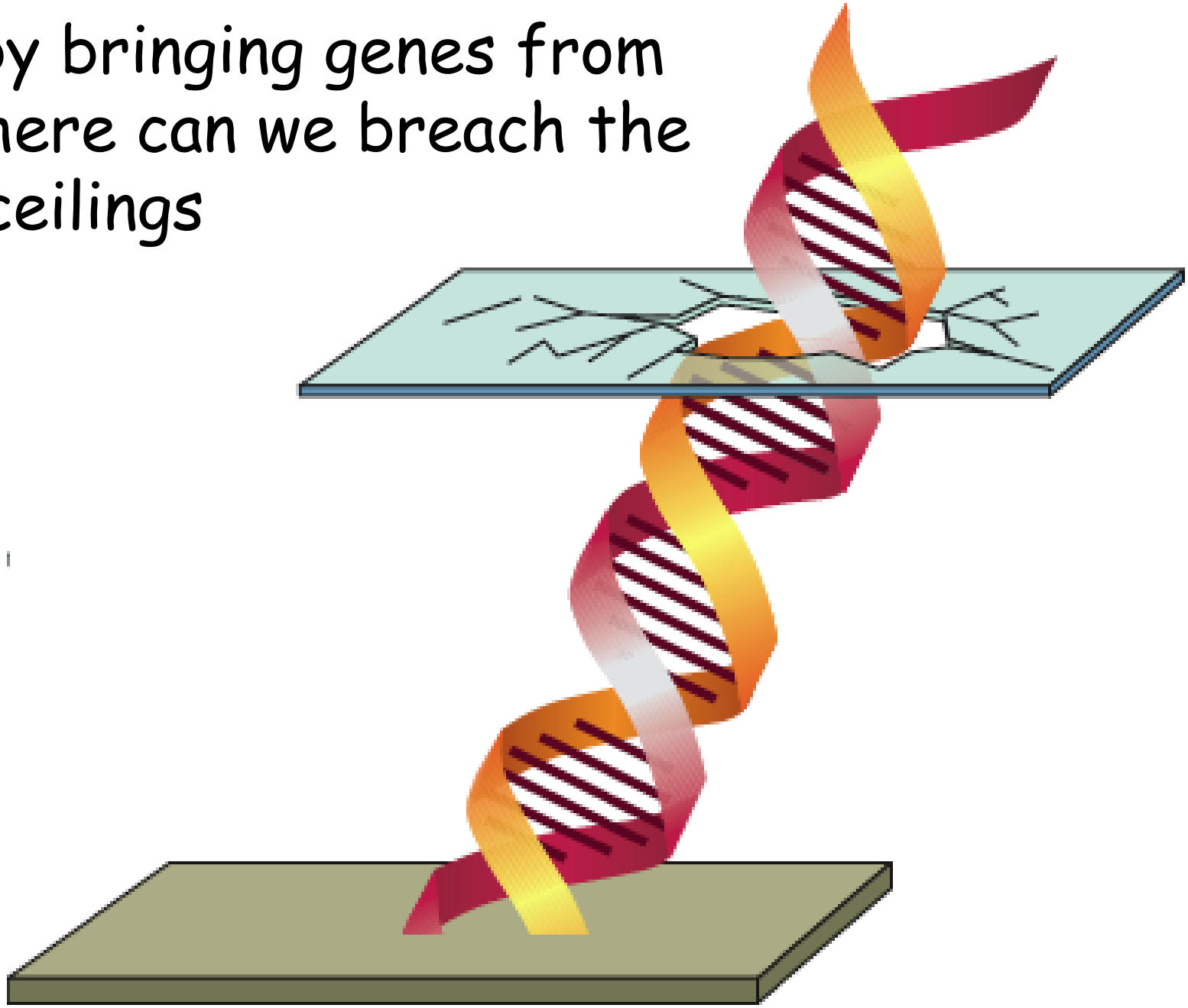
- grow special crops
- use wastes (straw)

3rd generation

- use algae as crops

What to do? These crops have not been domesticated for biofuels

Only by bringing genes from elsewhere can we breach the glass ceilings



To get rapid domestication of biofuel crops

Can you breach the yield barrier?

Sugarcane breeders reached an asymptote

Wu & Birch - Plant Biotech J 5:109, 2007 engineered

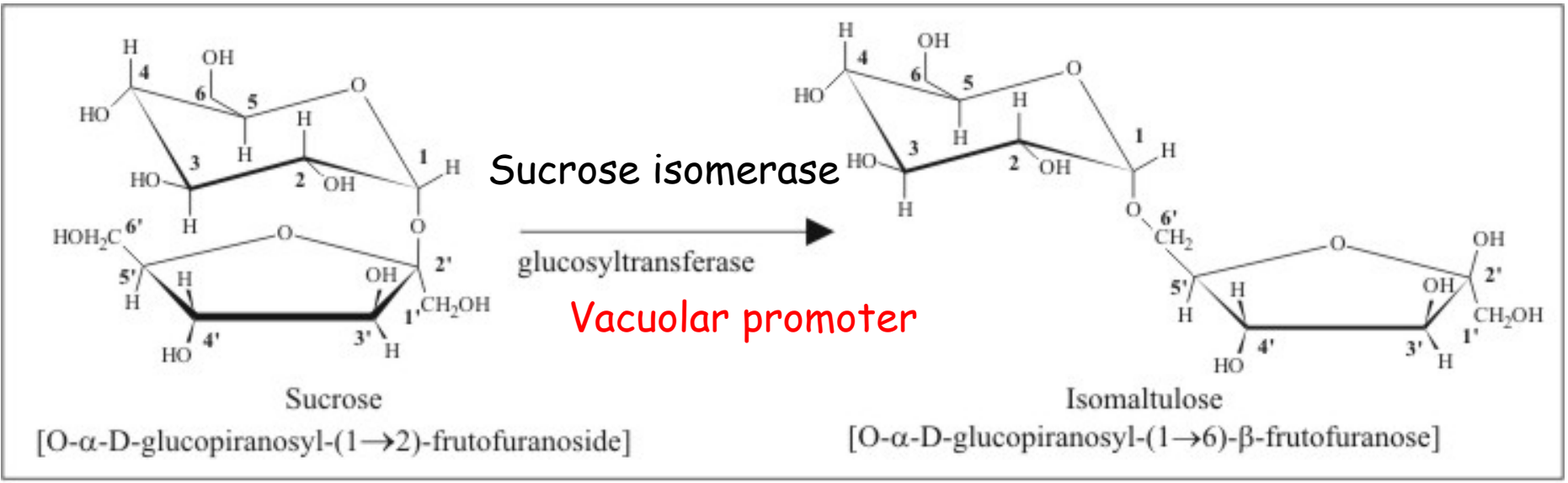


Figure 1. Conversion of sucrose into isomaltulose by glucosyltransferase.

Accumulates as much isomaltulose as sucrose

Sucrose constant = doubled yield

Problem: yeast do not metabolize isomaltulose

Need a gene for yeast - find or shuffle

Oilseed rape is favored for temperate climes

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 methyl-transferase

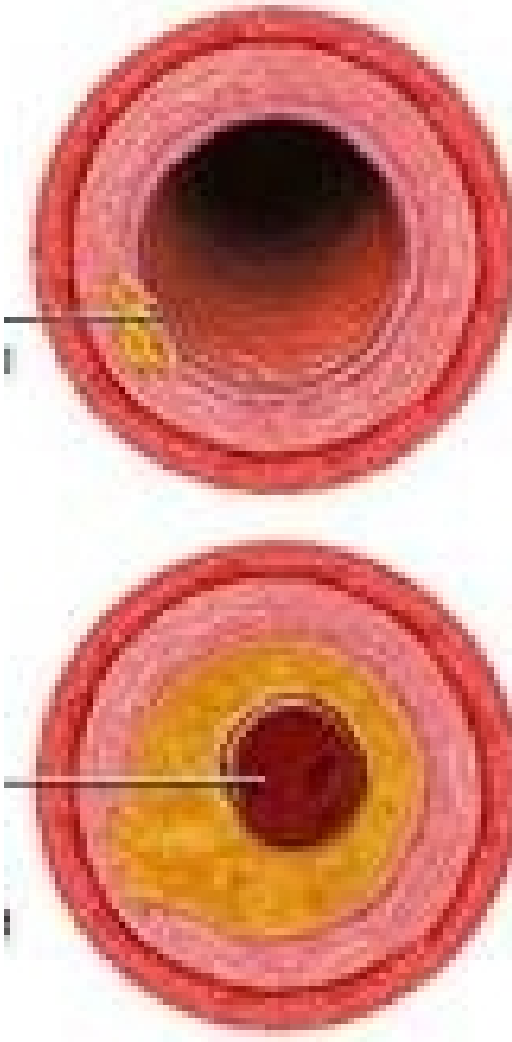
methylates halides to methyl halides (MeBr)
methylates bisulfides to methanethiol
(goes to H_2SO_4 to acid rain)
TDNA disruptive insertion in related *Arabidopsis HOL (harmless to ozone layer)* gene reduced MeBr >99%
To meet intent of methyl bromide ban and reduce acid rain, must cultivate only transgenic oilseed rape with this gene suppressed; non-transgenic should be banned

Palm oil makes poor biodiesel - congeals at low temperatures

Must catalytically crack it - or mix

Needs shorter chain length
- antisense elongases

Needs more mono un-saturation
- engineer desaturases



Such engineering = in non-cholesterogenic "palm-olive" oil

Area needed to replace 15% of USA transport fuels USA

Crop	Oil yield (l/ha)	area needed (M ha)	% of existing arable
Maize	172	462	178
Soybean	446	178	67
Oilseed rape	1,190	67	42
Jatropha	1,892	42	13
Oil palm	5,950	13	7.2
Algae/cyanobacteria[a]	59,000	1.3	1.3
Algae/cyanobacteria[b]	137,000	0.6	0.6

[a]containing 30% oil [b]containing 70% oil

Calculated from Chisti, Biotech. Adv. 25:294-306, 2007

The first generation

not sustainable in medium term

The second generation

Using agricultural wastes

lignocellulosics

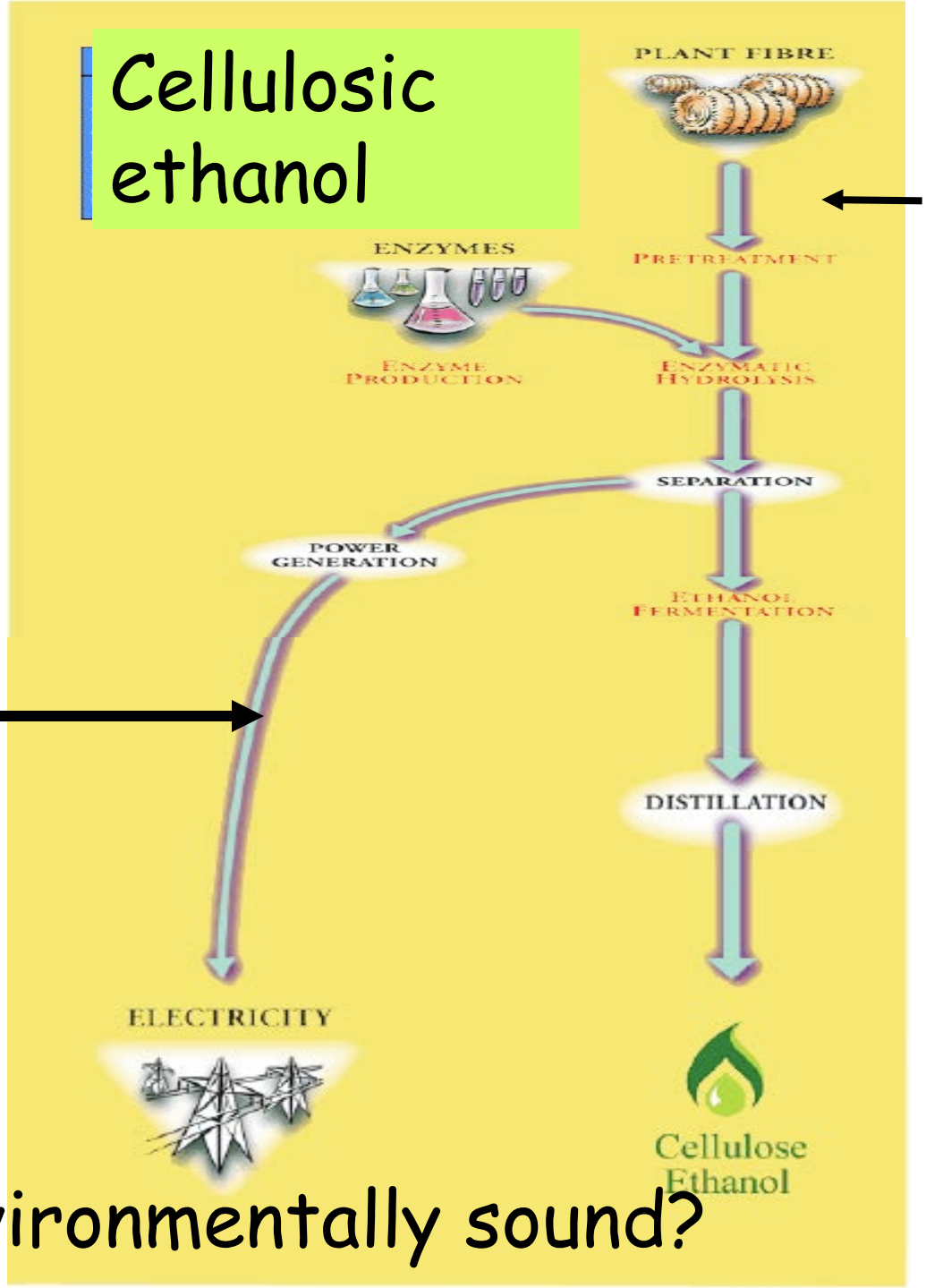
Cultivating biofuel dedicated crops

perennial lignocellulosics

perennial oilseeds

Today 2nd generation, Thurs. 3rd generation

Cellulosic ethanol

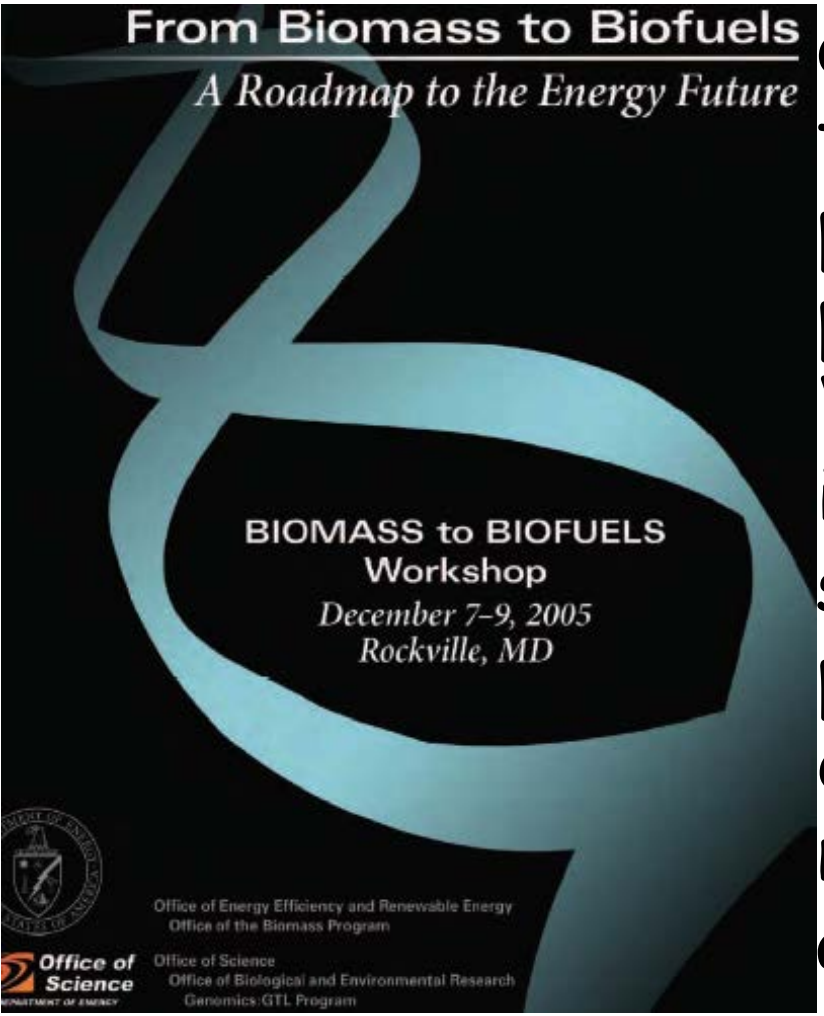


Acid, Heat

Air pollution

Is this environmentally sound?

Approach: optimize chem process/ not plants

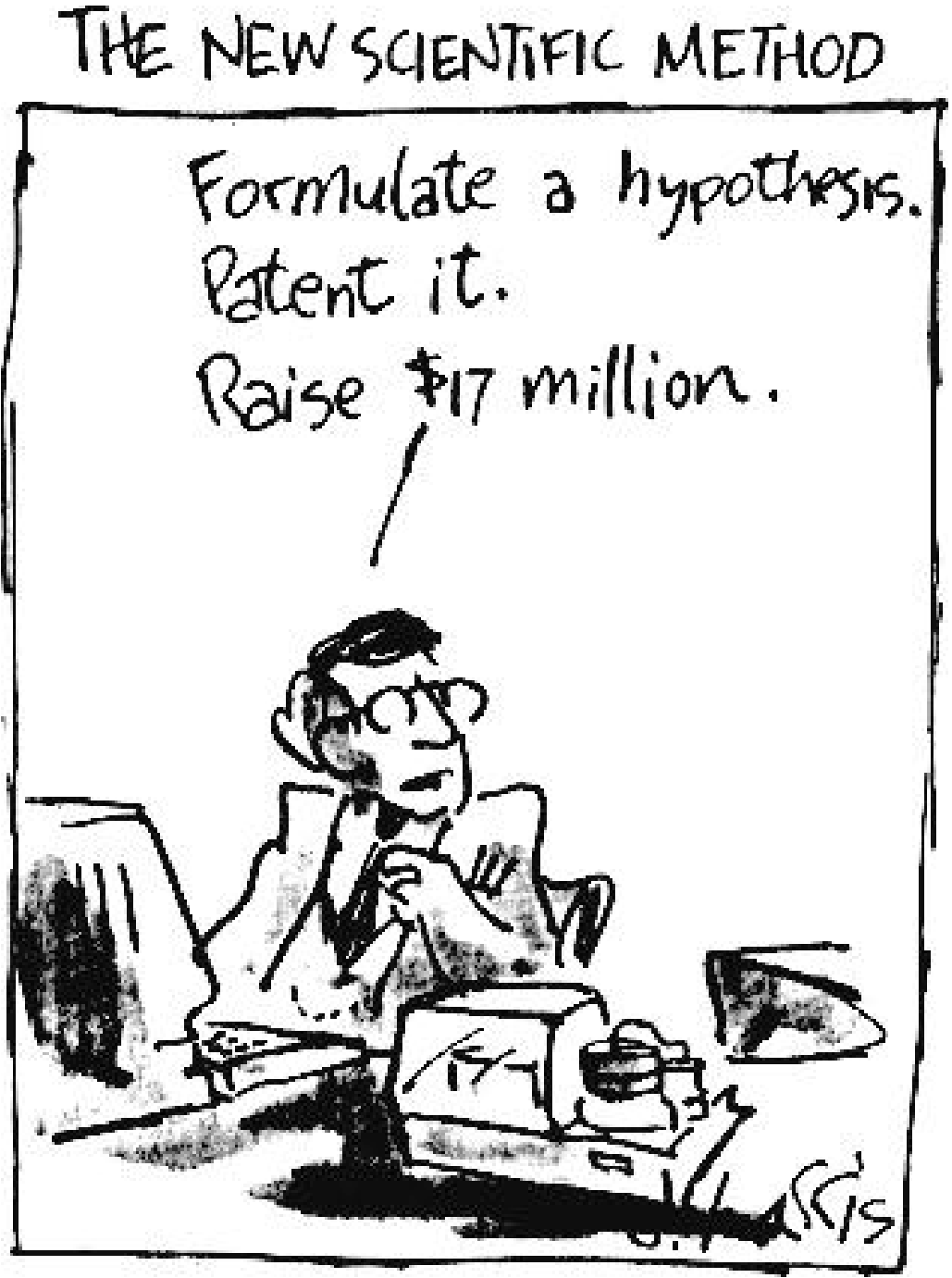


Executive Summary says:

"The key to a new biofuel industry based on conversion of cellulose (and hemicellulose) to ethanol is to understand plant cell wall chemical and physical structures.

With this knowledge, innovative energy crops specifically designed for processing to biofuel can be developed concurrently with new biology-based treatment and conversion methods."

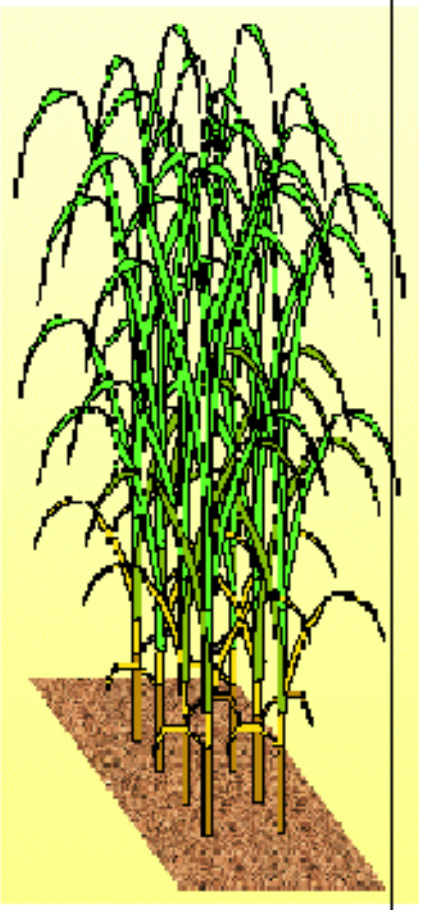
The old scientific method is not being used in biofuel R&D



The new method is being used - hard to distinguish between data and hype

Harvesting perennial Miscanthus

http://www.regensw.co.uk/images/miscanthus_harvesting.jpg



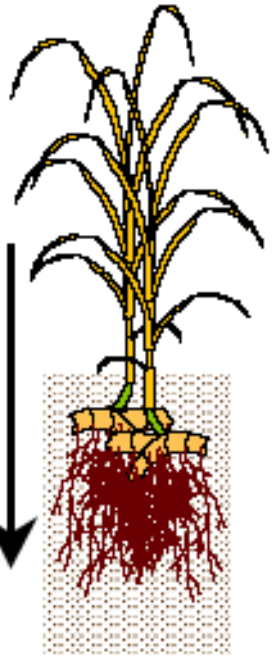
SPRING/
SUMMER

Mineral nutrients

Translocation from rhizomes to growing shoot

FALL

Mineral nutrients



Translocation to rhizome as shoot senesces

WINTER

Lignocellulose dry shoots harvested, nutrients stay in rhizomes

Courtesy of Dr. Steve Long, 2005

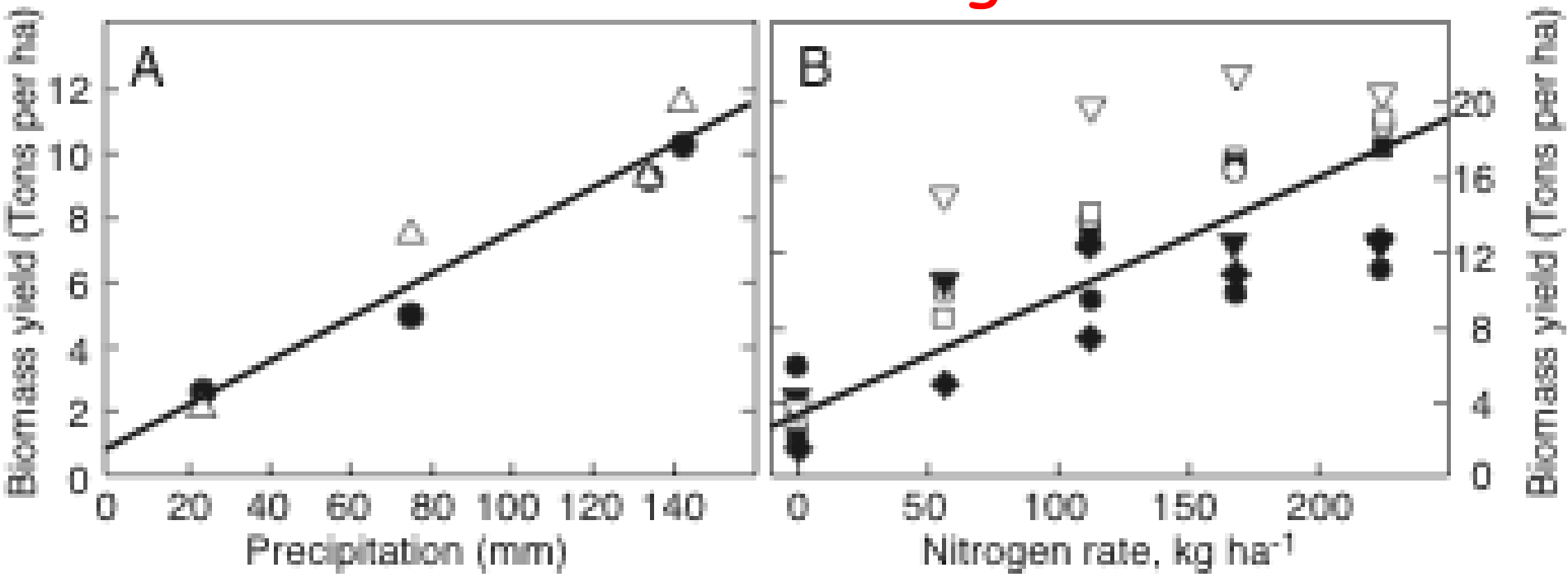
Multi-cut Switchgrass in Italy

www.switchgrass.nl/ photo_gallery.htm

Switchgrass does not defy the law of conservation of matter - grows best with

water

nitrogen fertilizer



Data of Lee et al. and Muir et al, collated in Gressel, "Genetic Glass Ceilings, Hopkins, 2007

The non-degraded switchgrass residue is burnt - energy for process

Contains 5-10% ash, >60% of ash=silica
On burning releases 50% more non-precipitable silica than coal*
Same with sugarcane bagasse/other grasses
Rice has the highest silica content of grasses
How dangerous is burning rice straw?

*Blevins, L.G., and Cauley, T.H. (2005) Fine particulate formation during switchgrass/coal cofiring. *Journal of Engineering for Gas Turbines and Power-Transactions of the ASME* 127, 457-463

Silicon not a required element for plants

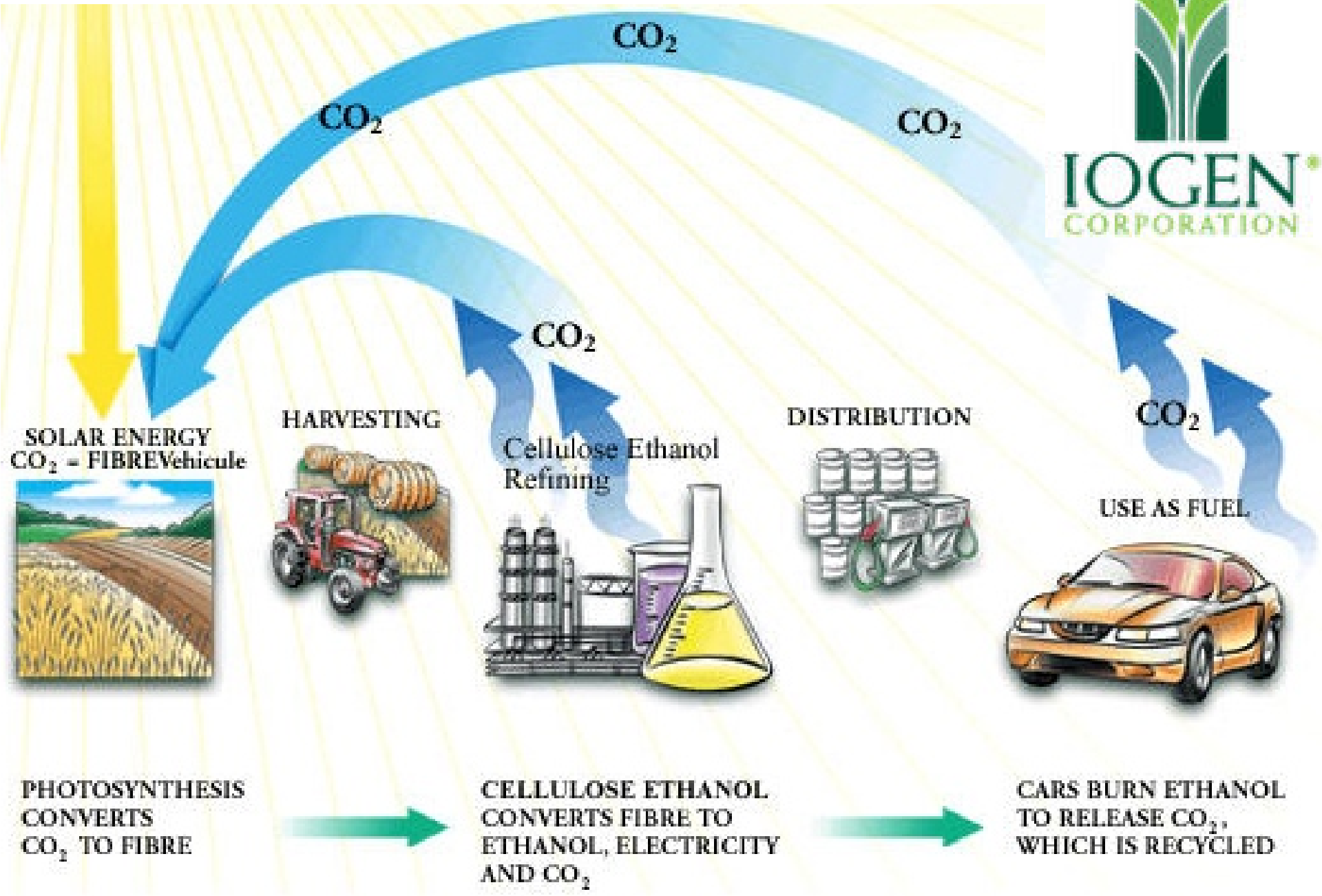
small amounts may be useful

but not the high amounts in many grasses, including sugarcane

Silicon transporters being discovered in plants

antisense/RNAi to lower levels?

Can grasses be turned into fuel?



Process:

Heat + acid pre-treatment (delignification)

Enzymatically digest cellulose to sugars

Ferment sugars to ethanol

But half of cellulose is unavailable

208 kg ethanol/ton straw

Claim: with present technology - Canadian wheat straw could provide ethanol for almost all Canadian automobiles

Can less heat / less acid be used if straw modified?

The higher the lignin content the lower the digestibility

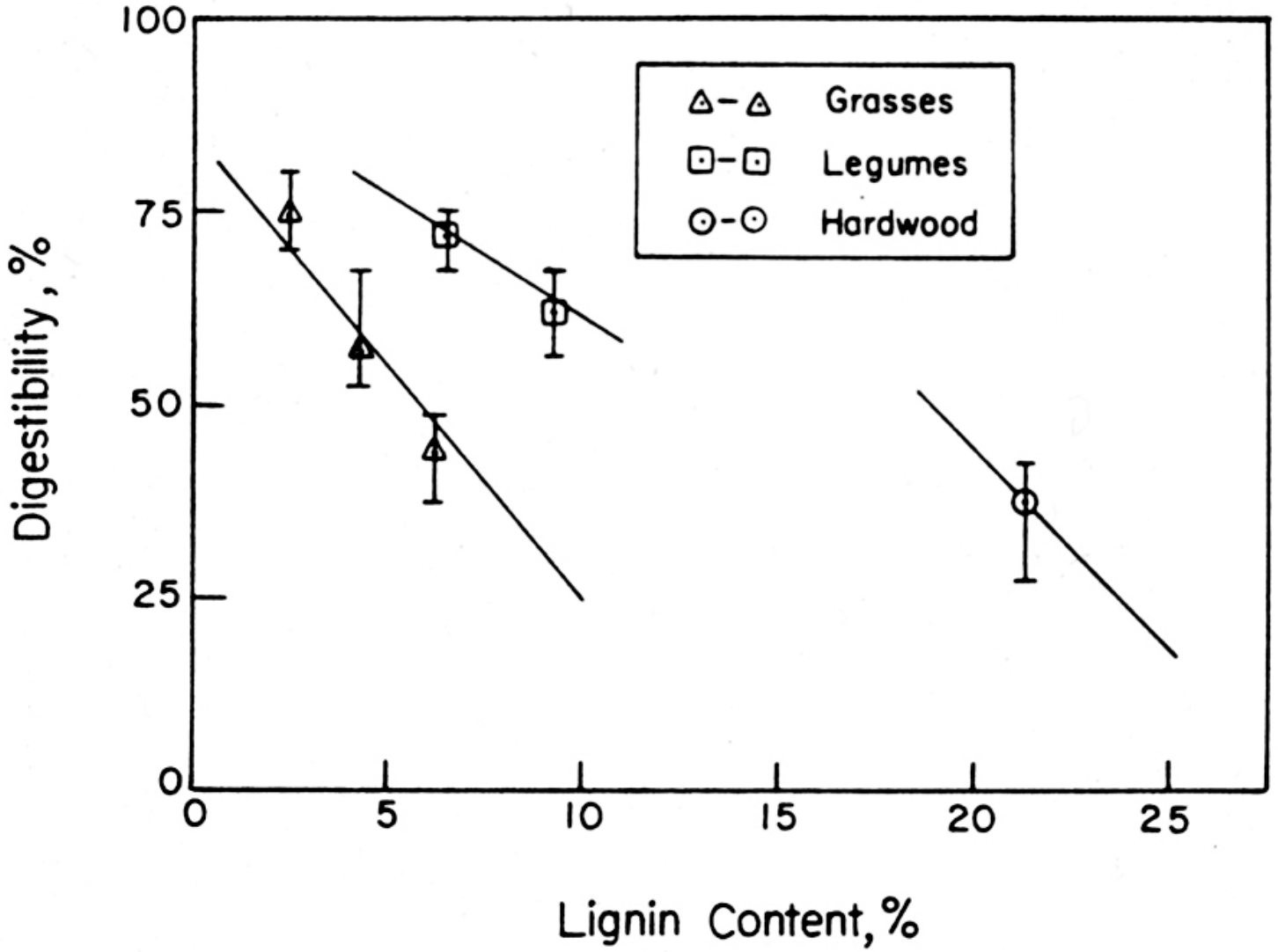
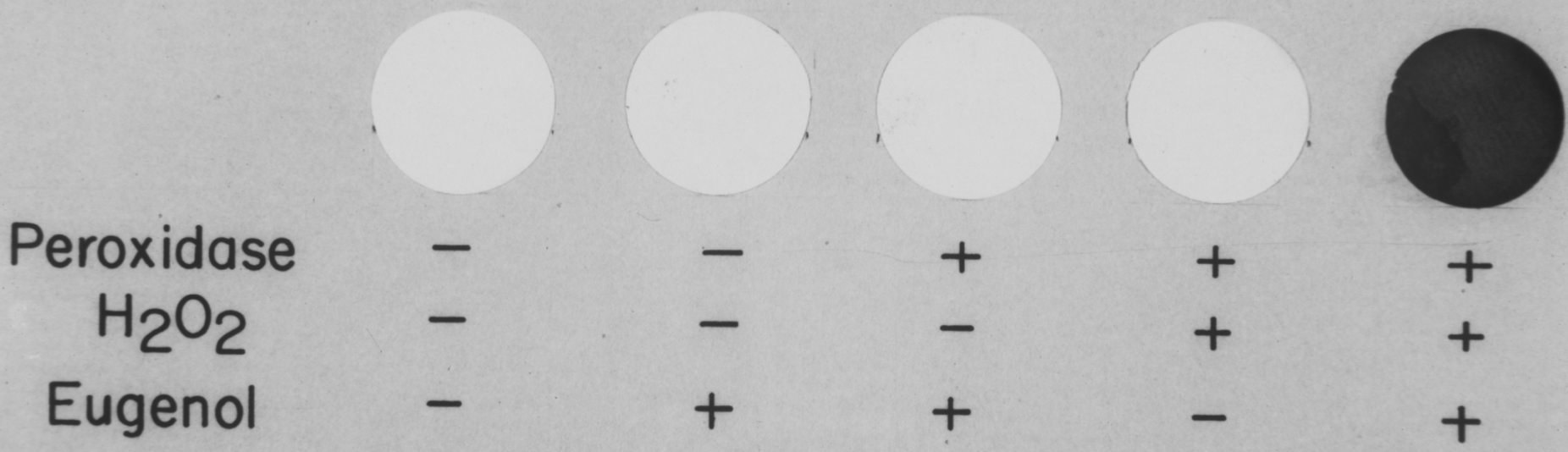


Fig. 6. Relationship between the dry matter digestibility and lignin content

Is lignin really the culprit?

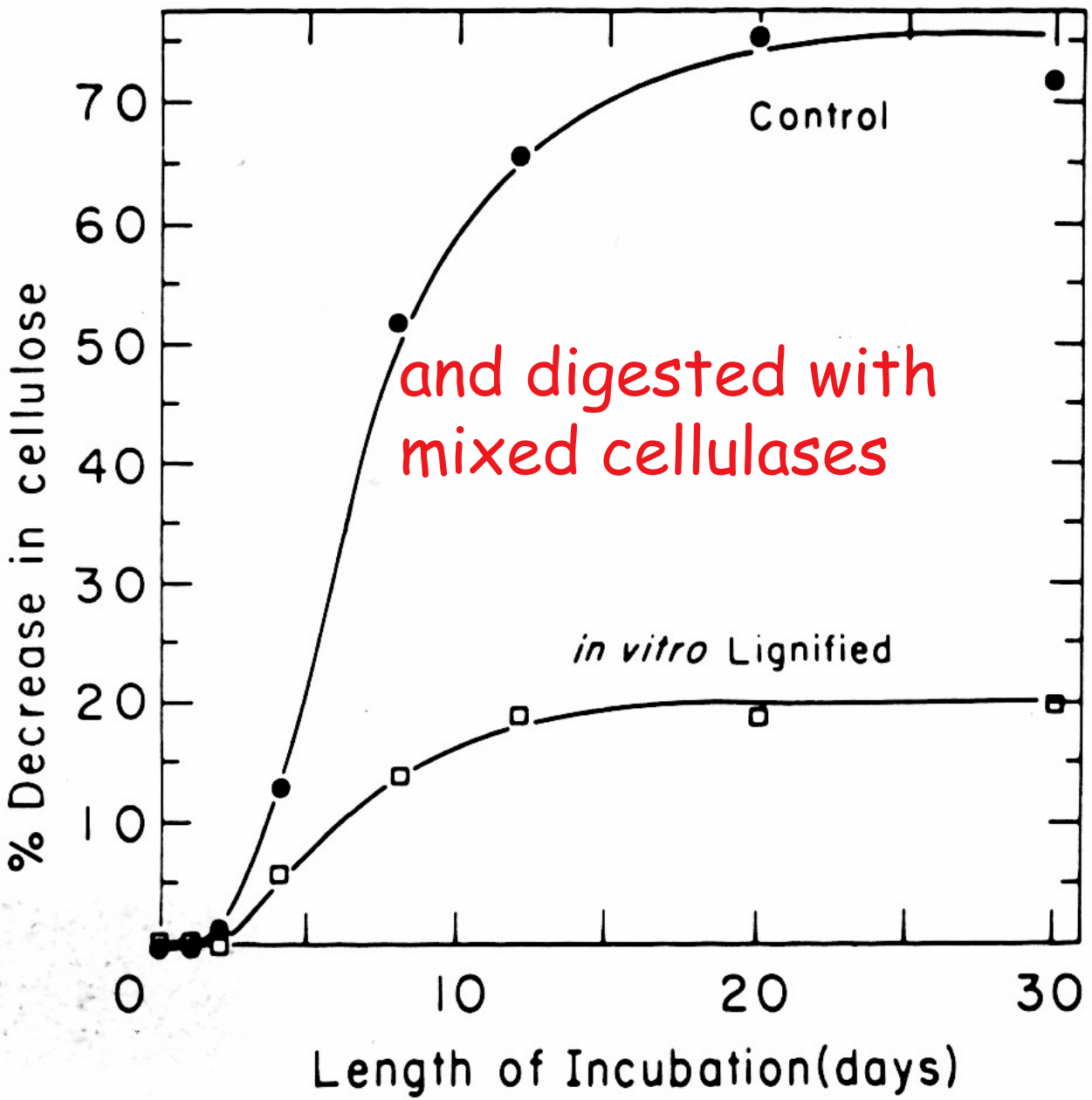
Cellulose can be artificially lignified



Stained for lignin with phloroglucinol

From: Gressel et al., Plant Sci. Lett., 32:349-353, 1983

Cellulose artificially lignified



and digested with mixed cellulases

The more lignocellulose is delignified the greater the digestibility by cellulases

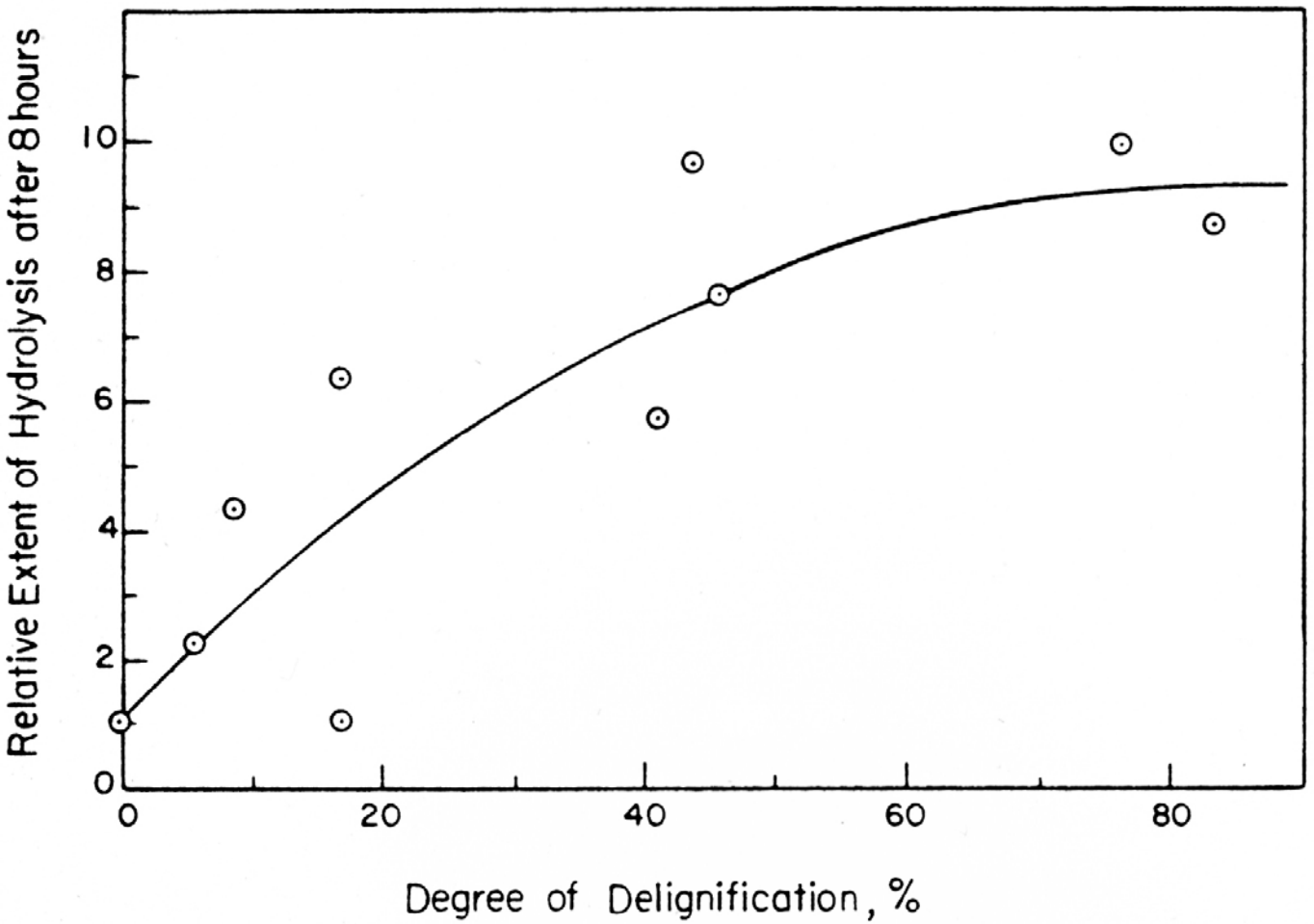
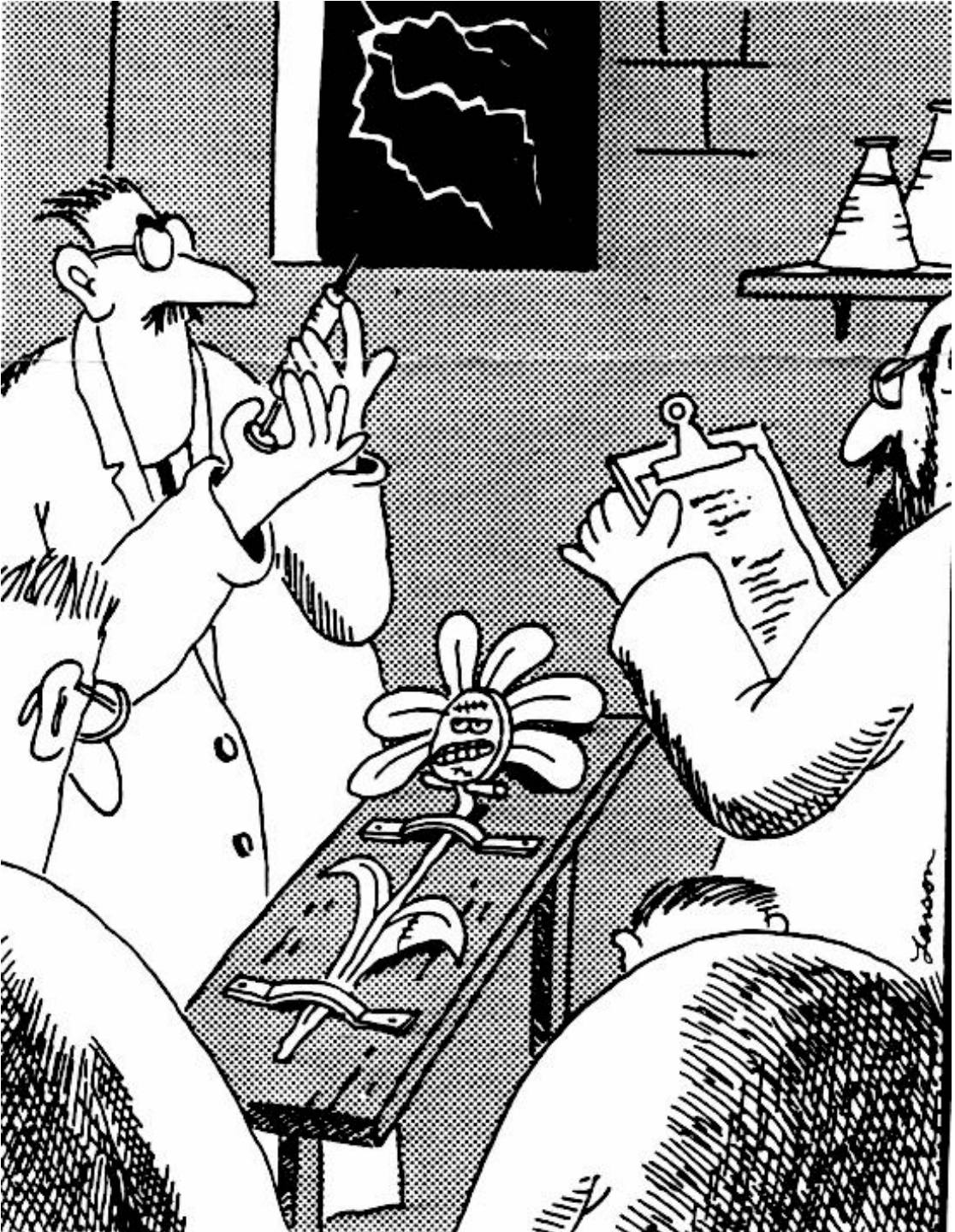


Fig. 5. Relationship between the extent of delignification and the hydrolysis rate



The solution:

Modify straw for:

-less lignin

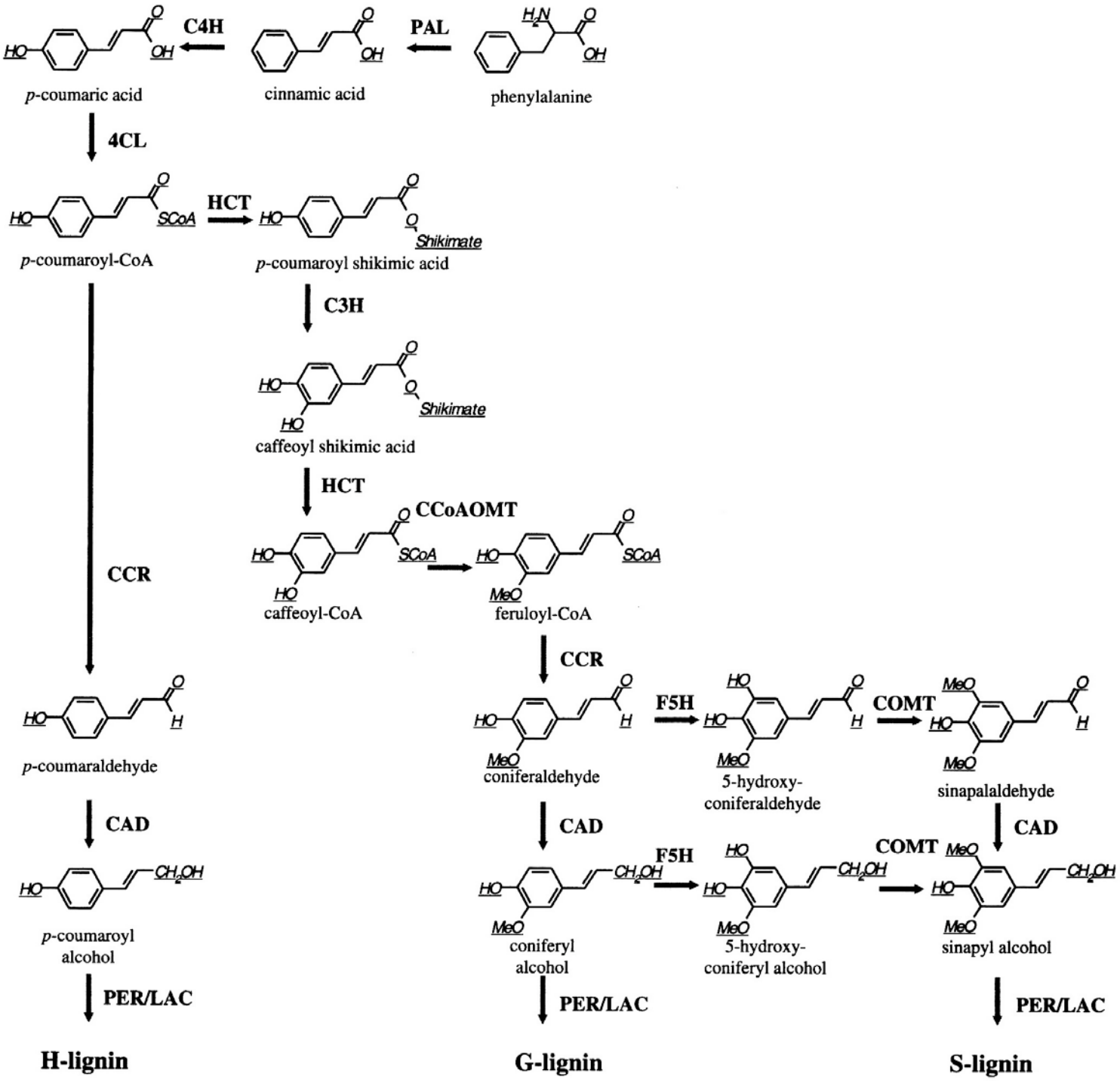
or

-modified lignin

or

- more cellulose

Should reduce the acid/heat requirement, add to yield


C4H
PAL
p-coumaric acid
cinnamic acid
phenylalanine
4CL
HCT
p-coumaroyl-CoA
p-coumaroyl shikimic acid
C3H
caffeoyl shikimic acid
HCT
CCoAOMT
caffeoyl-CoA
feruloyl-CoA
CCR
p-coumaraldehyde
CAD
p-coumaroyl alcohol
PER/LAC
H-lignin
CCR
F5H
COMT
coniferaldehyde
5-hydroxy-coniferaldehyde
sinapalaldehyde
CAD
F5H
COMT
CAD
coniferyl alcohol
5-hydroxy-coniferyl alcohol
sinapyl alcohol
PER/LAC
PER/LAC
G-lignin
S-lignin

There are many key enzymes/genes known

Rice Gene[a]	type	No. copies identified	Sequence identity (%) barley	wheat	maize	dicot[b]
PAL (AK067801.1)	FL-cDNA	at least3[c]	86	85	86	<76
C4H (AK104994.1)	FL-cDNA	at least 2	89	89	87	<80
C3H (AK099695.1)	FL-cDNA	at least 2	ni	89	79	<80
4CL ((AK105636.1)	FL-cDNA	at least2	83	ni	76	<80
CCoAO (AK065744)	FL-cDNA	at least 3	ni	93	90	<82
F5H (AK067847)	FL-cDNA	at least 2	ni	ni	ni	LS
COMT (AK061859.1)	FL-cDNA	>1	71	86	87	LS
CCR (AK105802)	cDNA	at least 3	88	85	90	<75
CAD (AK 104078)	FL-cDNA	at least 4	ni	ni	83	<71

The sequences are known

Use RNAi or antisense technologies

generate many transformants

will suppress to varying levels

screen optimal suppression/modification

Less lignin should = higher grain yield

Despite common suggestions / myth:

no correlation between lignin and strength

No reason to expect increased lodging if lignin slightly modified and / or reduced by a few percent

More cellulose

Engineer over-production of the cellulose binding domain causes over-production of cellulose

Probably best - stack
lignin reduction/modification
cellulose over-production

Many failures with this approach

Voelker et al. Antisense Down-Regulation of 4CL Expression Alters Lignification, Tree Growth, and Saccharification Potential of Field-Grown Poplar, Plant Physiology, 154: 874–886, 2010

The´venin et al The Simultaneous Repression of CCR and CAD,Two Enzymes of the Lignin Biosynthetic Pathway, Results in Sterility and Dwarfism in *Arabidopsis thaliana*. Molec. Plant 4:70–82, 2011 (complete suppression)

Li et al. Bioethanol production using genetically modified and mutant wheat and barely straws. Biomass and bio-energy 35: 542-548, 2011 (down regulated cinnamoyl-CoA reductase – only)

Some successes
- down regulating cinnamoyl-CoA reductase

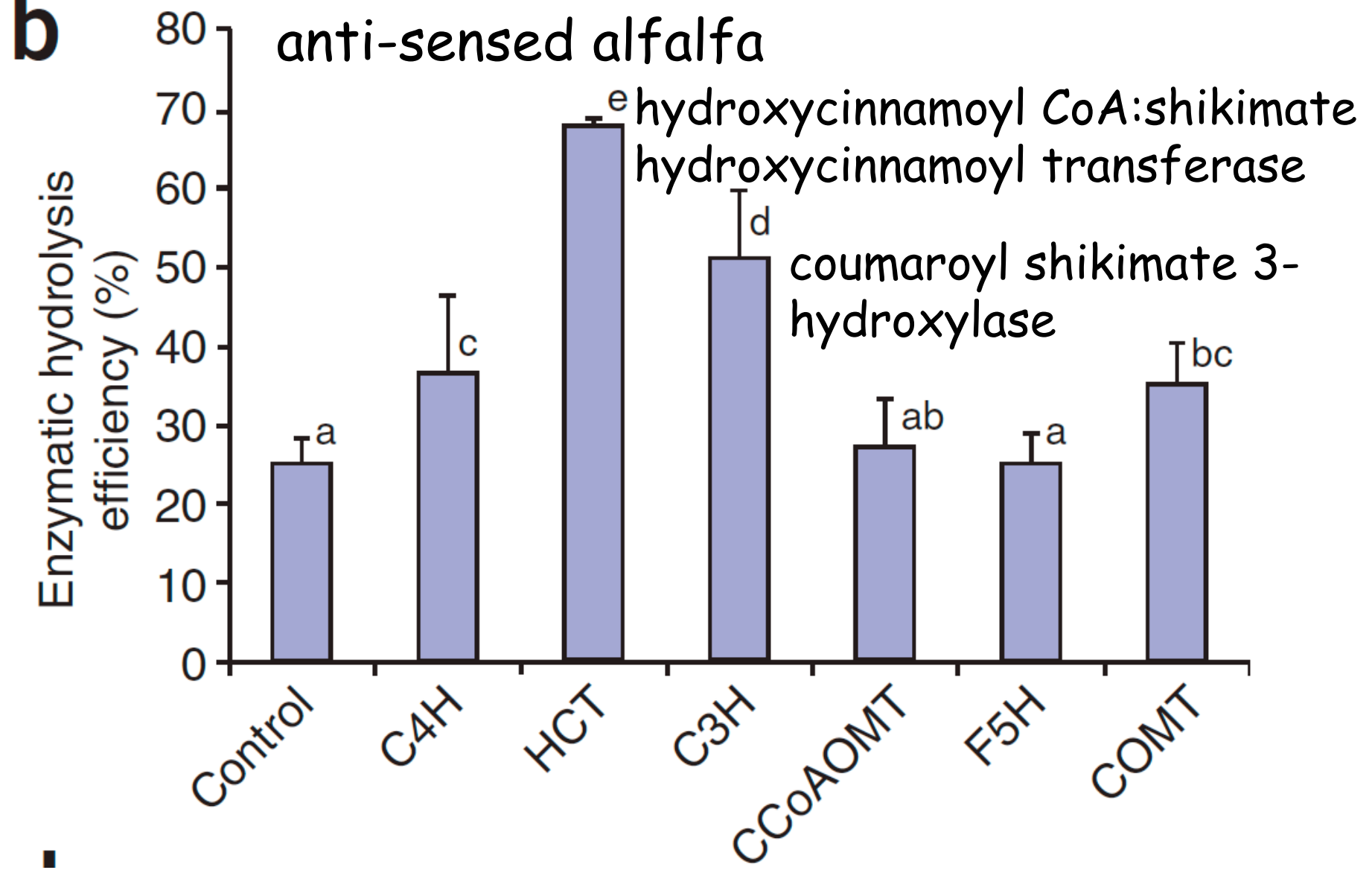
Release of sugars from rice straw mutants following heat and cellulase treatments

	Cell wall composition (%)			% degradation	
	Cellulose	Hemi-cellulose	Lignin	C_6 sugars	C_5 sugars
Wild type	49.9	24.7	25.4	18.5	4.3
RG 65	51.3	26.4	22.4	38.1	5.9
RG12	50.0	26.6	23.3	32.5	4.6

Condensed from Xie & Peng

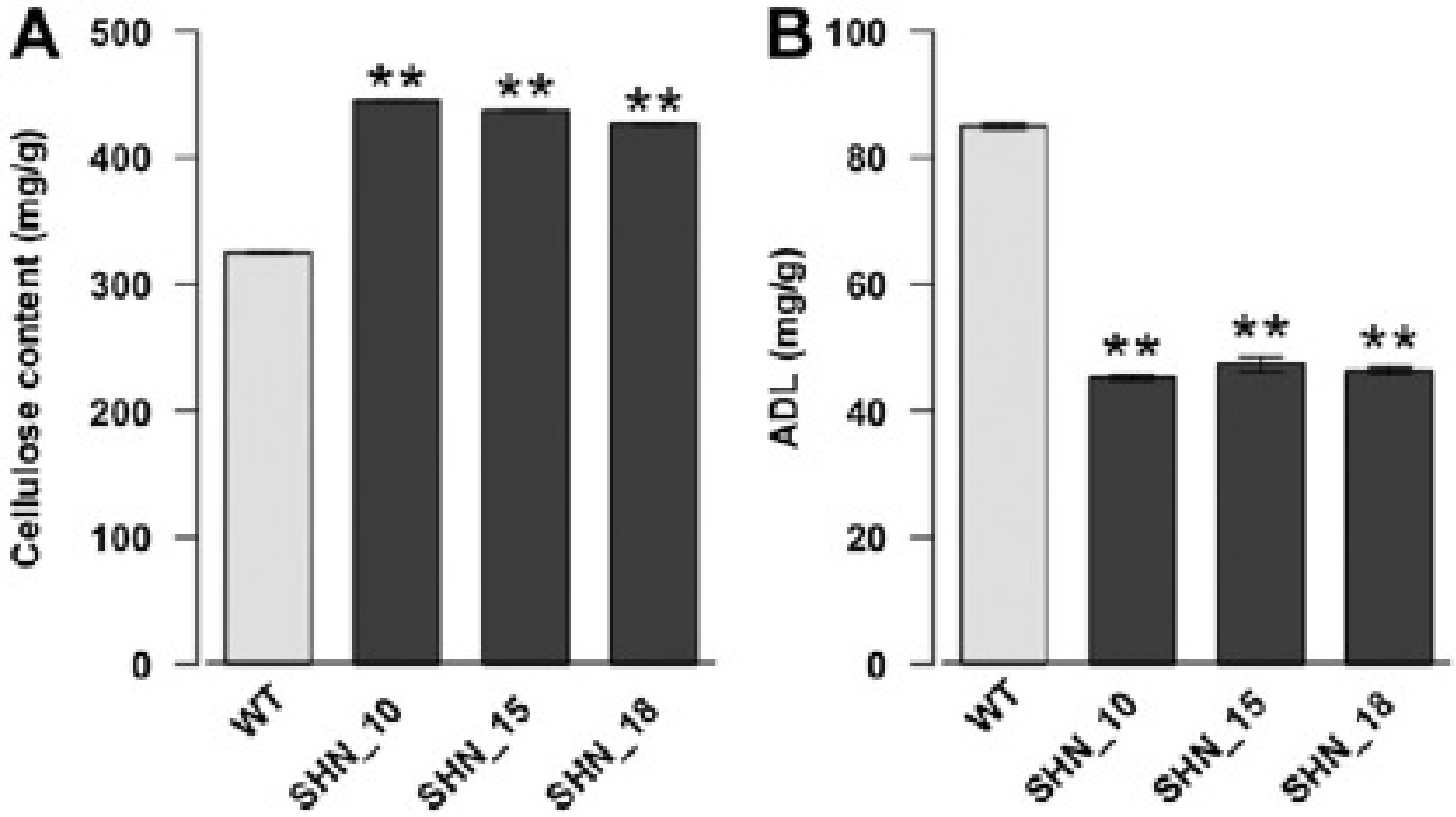
J. Integrative Plant Biology, **53**:143–150, 2011

Need small changes in lignin – not massive changes



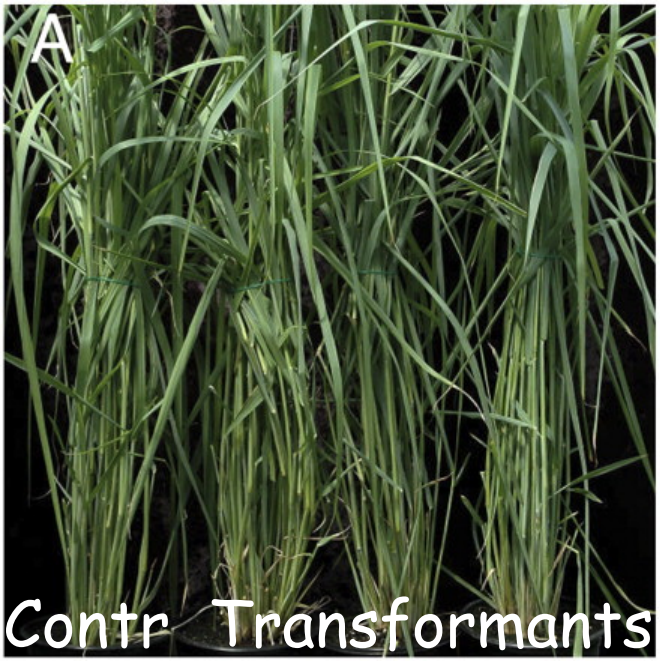
Chen & Dixon Lignin modification improves fermentable sugar yields for biofuel *Nature Biotechnology* 25: 759-761, 2007)

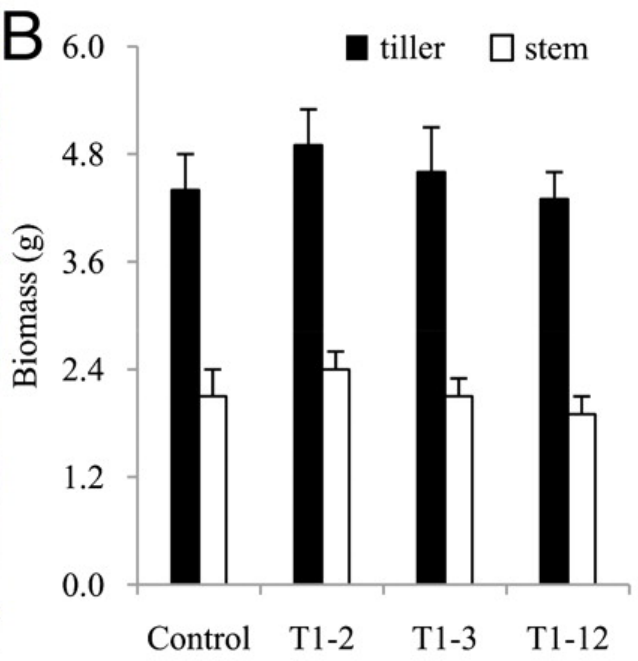
Transcription factor, SHINE increases cellulose and decreases lignin content in rice

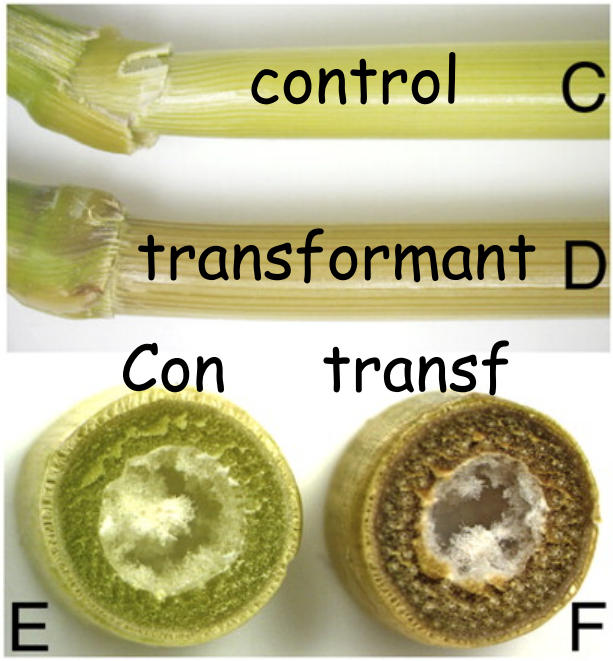


Ambavaram et al., Coordinated Activation of Cellulose and Repression of Lignin Biosynthesis Pathways in Rice. Plant Physiology 155:916–931, 2011

Phenotype of T1-generation transgenic switchgrass plants.



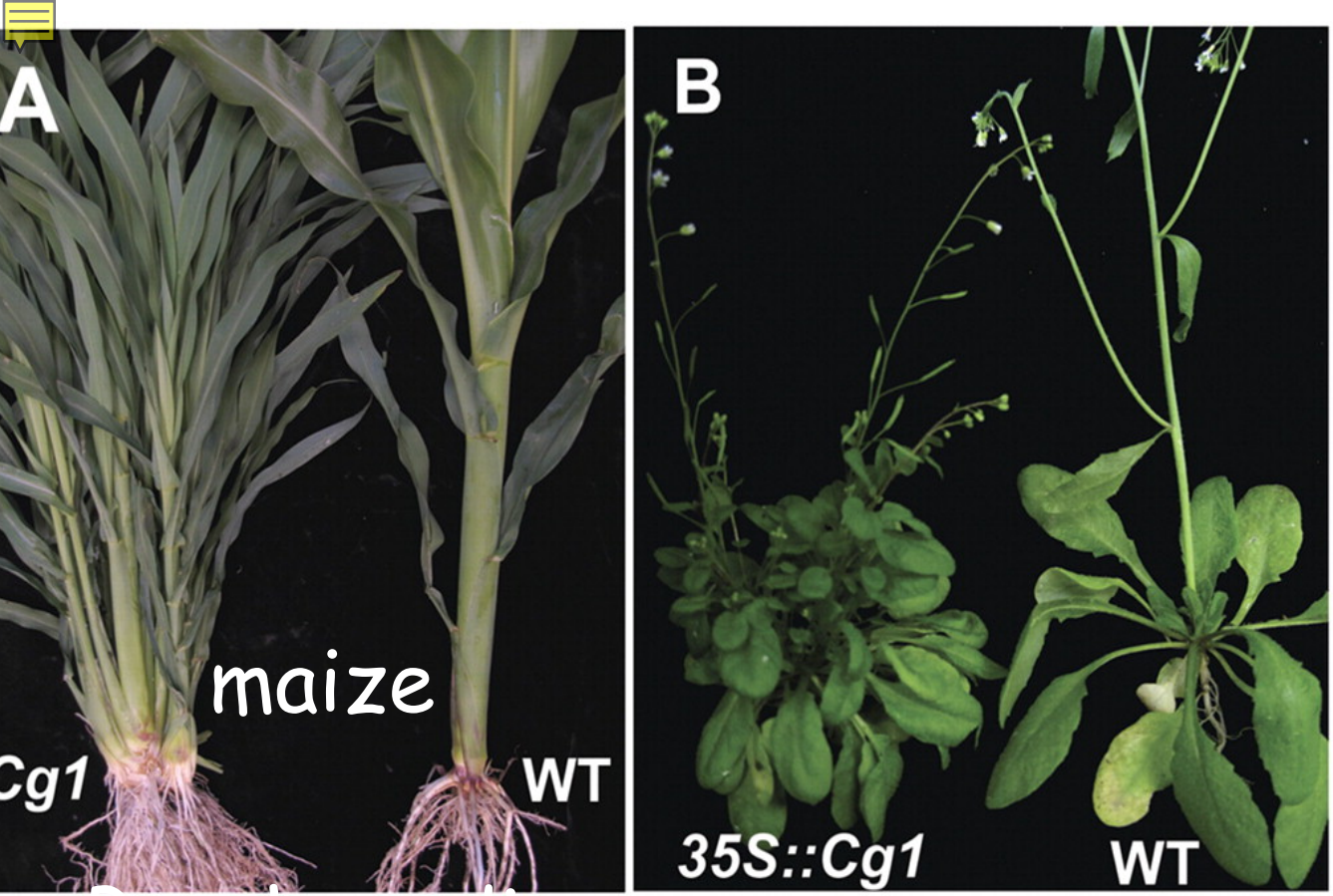


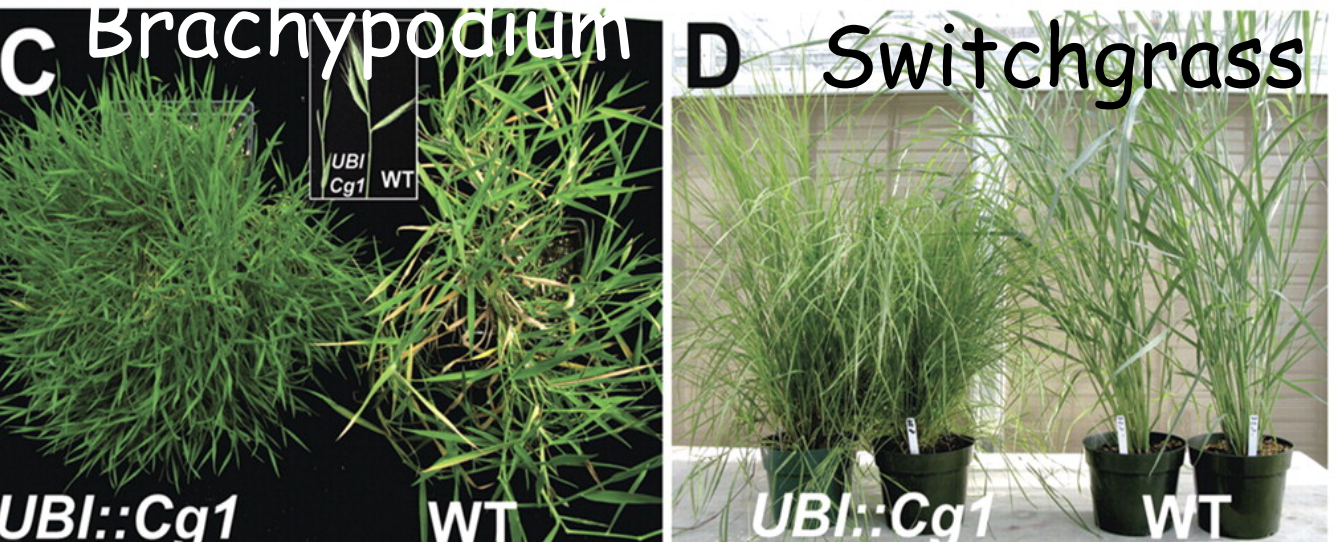


Down-regulation of caffeic acid O-methyltransferase decreases lignin, reduces the syringyl:guaiacyl ratio, improves forage quality, and, increases ethanol yield by up to 38% using conventional fermentation. requiring less severe pretreatment and 300–400% less cellulase (similar to brown midrib maize/sorghum)

Fu C et al. PNAS 2011;108:3803-3808

Overexpression of Cg1





Corngrass1 microRNA prevents flowering, improves digestibility, and increases starch content of switchgrass

Chuck G S et al. PNAS 2011;108:17550-17555

With "switchcanthus", land must be bought, dedicated to cultivation, watered, fertilized and harvested.

Straw is available "free" - a byproduct of grain production

World grain production (≈straw production)

wheat	rice	maize	sorghum	millet
million metric tons				
568	579	602	55	26

Total grain (total straw) ≈2,000 million tons

Source: FAO statistics I 2004

Why not use 2 billion T of free waste biomass?

Straw is not good for construction

Straw has negative economic/environmental value

- harbors pathogens if not burnt
  - -requires fungicides on next crop
- releases CO_2 if burnt
- binds nutrients while biodegrading
  - - requires more fertilizer - pollution

Straw has little value as animal feed or as a feedstock for bioethanol production.

- despite ca. 70% carbohydrate
- less than half digested

Can we turn straw into something valuable?

Maybe not into gold, but into bioethanol

Proposal:
Until Malthus arrives in developed world & until CO_2-free fuel sources available, use modified straw in:

Developed world:

Use technology for bioethanol

Developing world:

Use technology for ruminant feed

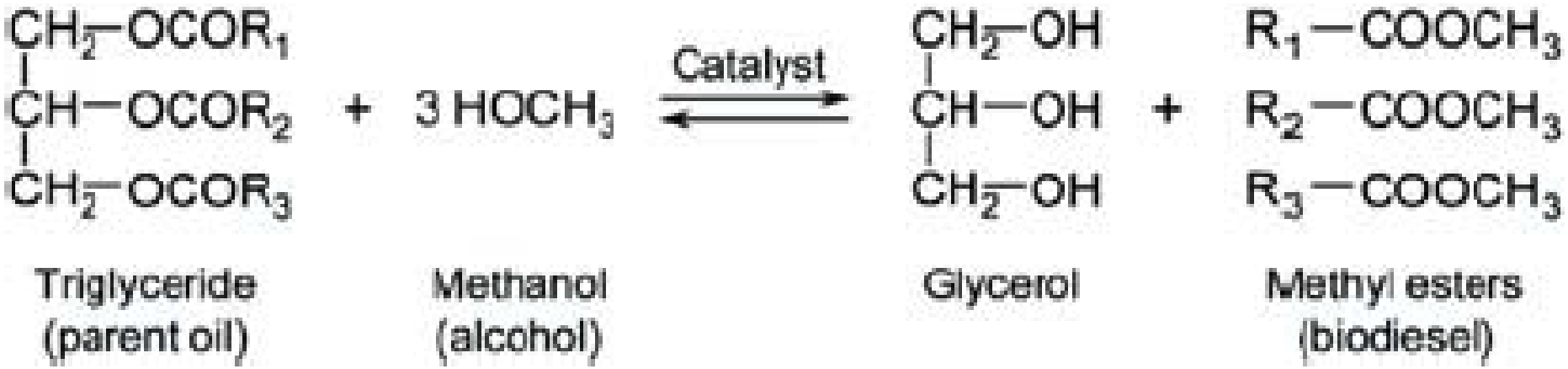
All users should get carbon credits

Is using straw waste sustainable?

Soil scientists say "no!"
-need organic matter in soil
-(but straw used to be burnt in Europe)

Most now agree - ok if 20% left in field

Biodiesel from various sources



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?

Info not in sites promoting *Jatropha curcas*

common plant names: Black vomit nut, Purge nut, etc.
common oil names: hell oil, oleum infernale, etc.

Toxins: Curcin (a toxalbumin) - similar to ricin
Phorbol esters - diterpenoids Alkaloids
skin tumor promoters

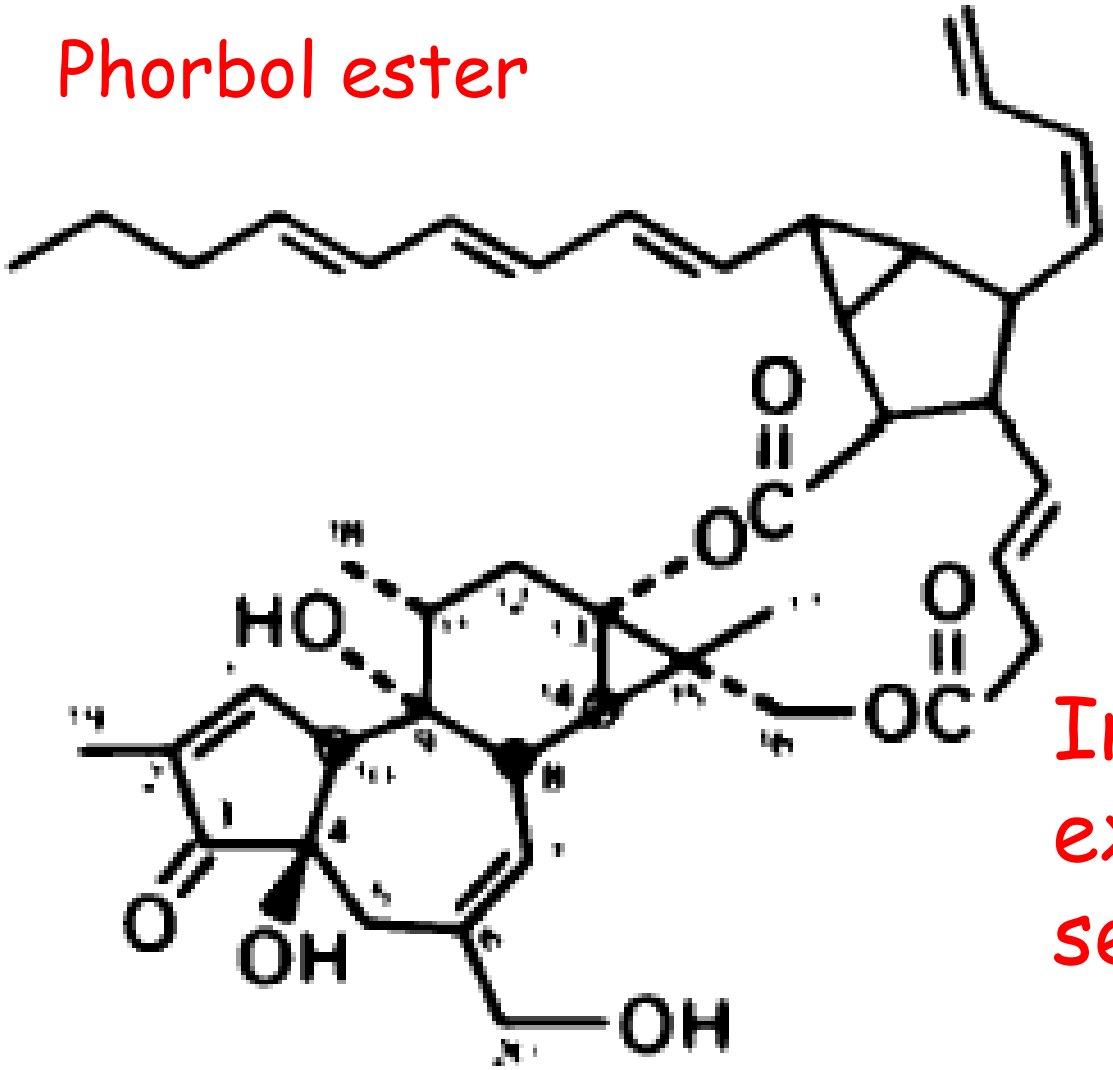
No antidote known

See: http://www.inchem.org/documents/pims/plant/jcurc.htm

Also: A case of Jatropha poisoning resembling organophosphate intoxication Clin. Tox. 44 337,2006

Could one release a transgenic crop with such components? What to do with toxic byproducts?

Phorbol ester



In the oil and expressed seedcake

Hirota et al. A new tumor promoter from the seed oil of *Jatropha curcas* L., Cancer Res 48(20): 5800-5804, 1988

Is "non-toxic"-Mexican *Jatropha* not toxic?

	curcin[a]	phorbol esters[b]	trypsin inhibitor[c]	phytate[d]	saponins[e]
3 *Jatropha* varieties (average)	102	2.39	20.3	8.9	2.2
"non-toxic"-Mexican *Jatropha*	51	0.11	26.5	8.9	3.4
soybeans (control)	<0.5	-	3.9	1.5	4.7

[a]measured 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?

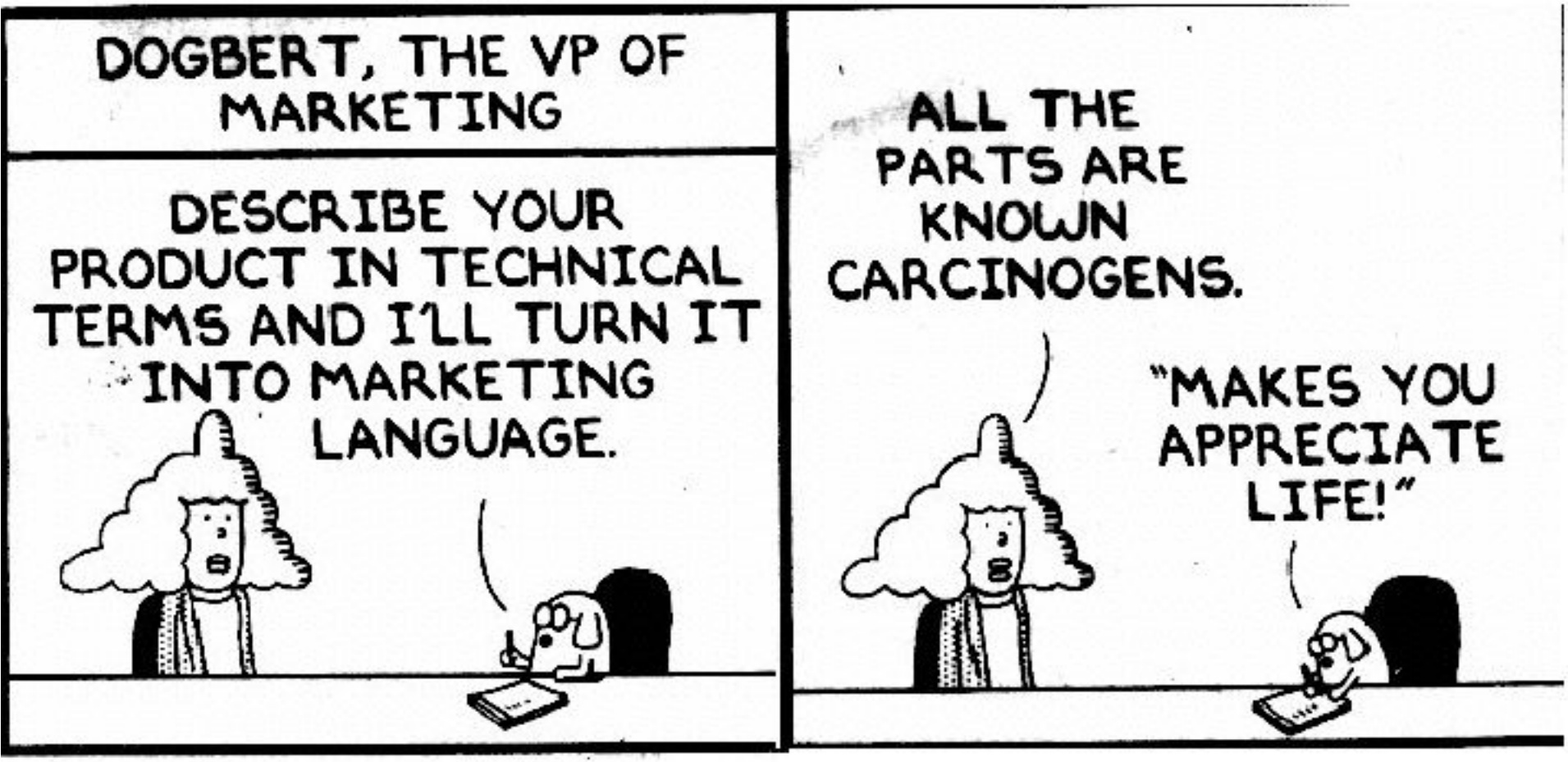
Websites suggest - Use residue as manure

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



Castor oil for biodiesel

Castor has similar problems as Jatropha

Seeds contain 0.2 to 3% ricin

1 mg/kg toxic
fill car with 50 liters (13 gallons diesel)
enough ricin byproduct to kill 3 people
at lowest content, 45 at highest

Not transgenic - no environmental impact studies needed - no regulatory scrutiny

Ricin protein "easy" to eliminate transgenically!

Two Oklahoma legislators introduced a bill to outlaw production and transportation of castor

Castor beans contain 50% or more oil for producing biofuels. They also contain high levels of ricin, a potent toxin that induces a slow, agonizing death marked by vomiting and diarrhea. Edible crop producers became concerned about ricin residues in fields, equipment, storage bins, and transportion.

Jatropha banned in Western Australia as "toxic to man and livestock"

Approach should be to ban the toxins – stimulate domestication

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 /ricin & agglutinin genes
  gene excision + RNAi
- RNAi terpene synthase to rid of phorbols
- RNAi pathways to other toxins/allergens
- better yield, oil content / quality

The engineered crop might then be safe to grow

Can reduce by breeding
- why not continue breeding?

Ricin production dominant
pollen from neighbors

RNAi/antisense dominant for non-production

Override pollen

Summary predictions:

- In the short term - biofuels will come from food crops
- In the medium term - biofuels will come from cellulosic wastes and algae
- In the long term - only algae; cellulosics to ruminant animals

Biofuel feedstocks will be transgenic

For more information

Available online at www.sciencedirect.com

ScienceDirect

Plant Science 174 (2008) 246–263

PLANT SCIENCE

www.elsevier.com/locate/plants

Review

Transgenics are imperative for biofuel crops

Jonathan Gressel [a,b,*]

Happy to send you a pdf

"A word to the wise is not sufficient - if it makes no sense

James Thurber

I hope I have made some sense today-

Thank you