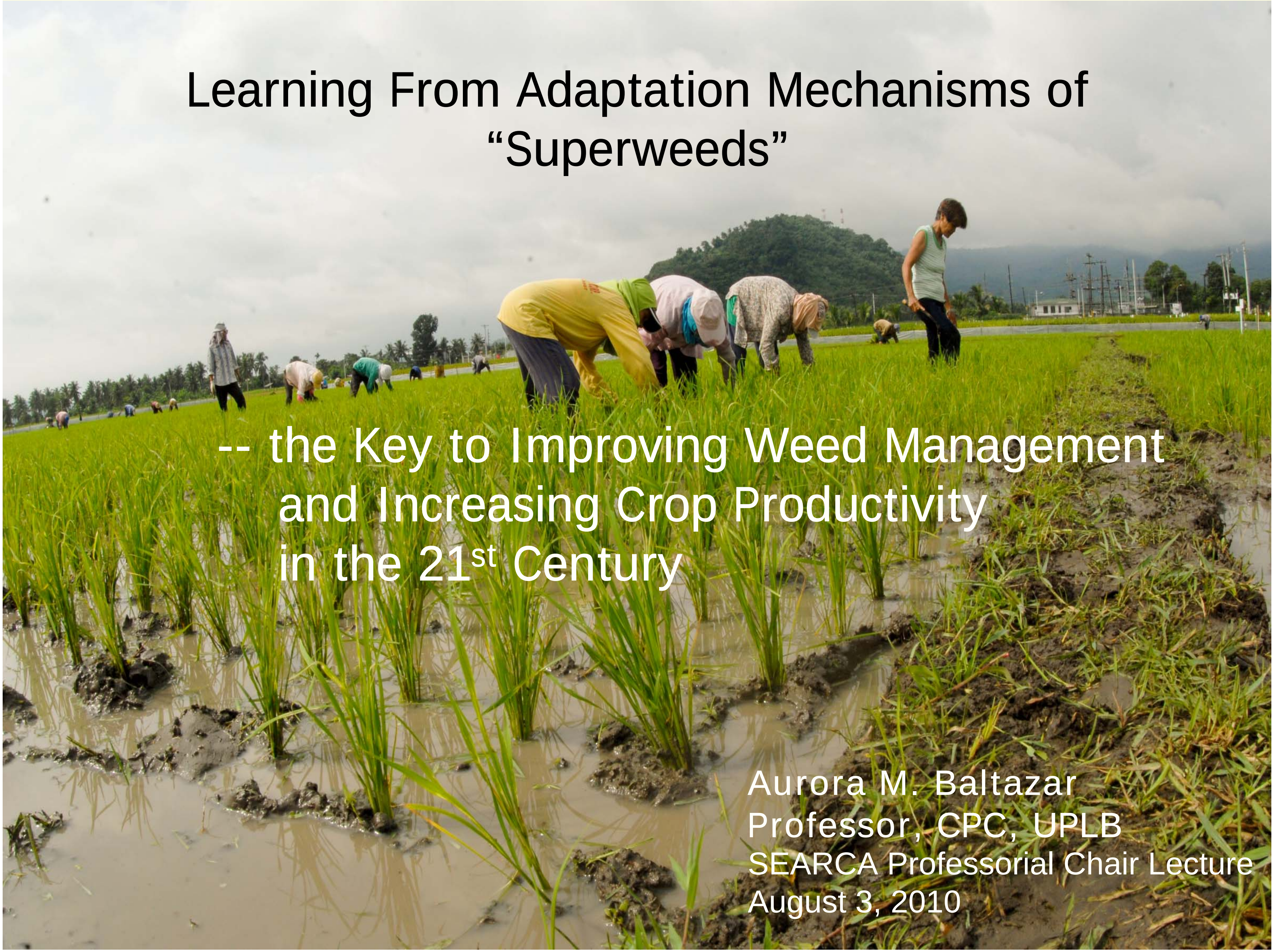


Learning From Adaptation Mechanisms of "Superweeds"

-- the Key to Improving Weed Management and Increasing Crop Productivity in the 21st Century

Aurora M. Baltazar
Professor, CPC, UPLB
SEARCA Professorial Chair Lecture
August 3, 2010

Annual cost ($) of crop pests (Klingman, 1975)

Pest	Losses	Control	Total	% of total
Diseases	3,152,815	115,000	3,267,815	27
Insects	2,965,344	425,000	3,390,344	28
Nematodes	327,335	16,000	388,335	3
Weeds	2,459,630	**2,551,050**	5,010,680	42

Onion – 3 handweedings/season
180 man-days; P50,000/ha/season
(Baltazar, et al, 2001)

TPR – 100-200 man-hrs/season
DSR – 300-400 man-hrs/season
(De Datta and Moody, 1982)

4 MANILA BULLETIN THE NATION'S LEADING NEWSPAPER News

RP farmers wary of superweeds threat in

By MARVYN N. BENANING

The spread of superweeds that have immunity to the herbicide glyphosate in the US has become a matter of concern to Filipino farmers cultivating biotech corn for years.

Bacillus thuringiensis (Bt) corn has been planted in more than 400,000 hectares of land in the Philippines and the area for the genetically-modified (GM) corn is increasing rather on account of the high output and less dependence on herbicides.

Bt corn was the first biotech crop approved for commercialization in the country.

The success of Bt corn was such that farmers took to it as duck do to water, with hectarage expanding since 2003.

With Bt corn, the output of yellow corn surged and farmers adapted the best practices for biotech corn, which promised to be herbicide-tolerant and needed less chemical fertilizers to grow.

Since the biotech corn requires less tillage, it represented a significant reduction in labor costs and an increase in incomes for farmers.

The same experience held true in the United States, where farmers cultivating soyabean, corn and cotton accepted biotech crops with superior yield and less reliance on herbicides.

Roundup Ready corn was one such crop. It was genetically engineered to tolerate Roundup, which is essentially glyphosate, a herbicide that was developed to kill weeds.

However, the use of Roundup Ready, with its herbicide resistant characteristics, also led to the rise of superweeds, which were initially discovered in Delaware in 2000.

Noted cardiovascular and thoracic surgeon Dr. Fernando Melendres says the rise of these superweeds is similar to the mutation of antibiotic-resistant strains of tuberculosis.

The widespread use of antibiotics led to the development of these antibiotic-resistant strains, he added.

Charina Garrido Ocampo, corporate affairs chief of Monsanto Philippines, says the company is fighting a prolonged battle with the superweeds, which the New York Times said was threatening to reduce yields for US farmers who now have to apply a variety of herbicides to stop the growth of the superweeds.

Ocampo stressed Monsanto has been pushing for the best practices to battle the superweeds, which war the company says is manageable. No less than 80 studies have been conducted by US scientists in finding the best way to eliminate the superweeds, she added.

Ocampo said this is the response of the company to a recent report of the US National Research Council (NRC) entitled "Impact of Genetically Engineered Crops on Farm Sustainability in the United States" issued last month.

While NRC confirmed that farmers who have adopted biotech crops have "experienced lower costs of production and obtained higher yields in many cases because of more cost-effective weed control and reduced losses from insect pests," it also warned of dire consequences if genetically-engineered crops and associated technologies are not managed prudently in the future.

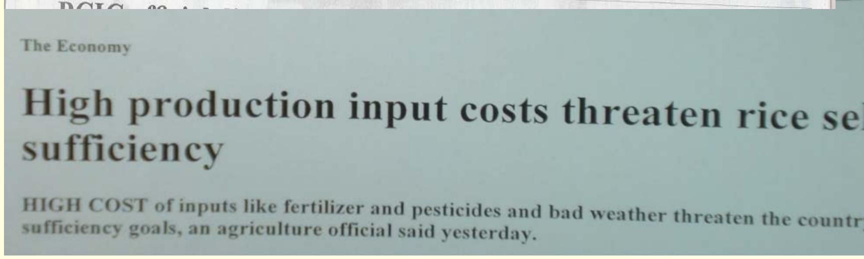
NRC said weed resistance to glyphosate is an issue that deserves attention not only by Monsanto, which manufactures glyphosate, but also by other agricultural stakeholders.

Monsanto said lyphosate continues to manage 300 different types of weeds and added that there is no single management method that can completely eradicate weeds.

On the part of Filipino corn farmers, fears are rife that their stands of Roundup Ready might be ruined by these weeds, leading to lower output and corollarily, lesser incomes.

NRC concluded that efforts must be undertaken to eliminate

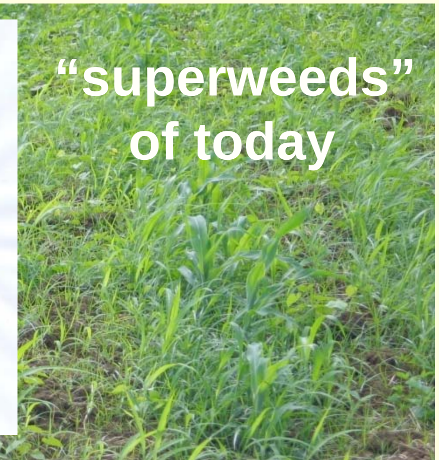
cide rotation, herbicide application sequences, and tank-mixes of more than one herbicide; herbicides with different modes of action, methods of application, and persistence; cultural and mechanical control practices; and equipment-cleaning and harvesting practices that minimize the dispersal of herbicide resistant weeds.



The Economy

High production input costs threaten rice sel
sufficiency

HIGH COST of inputs like fertilizer and pesticides and bad weather threaten the country
sufficiency goals, an agriculture official said yesterday.



Single season, direct-removal approaches –
herbicides, handweeding, interrow cultivation
"Putting out the same fire every cropping season"
Tedious, expensive, increase production costs
Major weeds of yesterday are "superweeds" of today
Need for innovative approaches to reduce direct removal inputs
To be able to do this, need deeper understanding of
"weediness" and weed survival and adaptation mechanisms

Topic outline

- Weediness: Ability to adapt, resist control, and multiply:
  Why major weeds of yesterday are still "superweeds" of today

- A tale of two "superweeds" – adaptation mechanisms
  - Barnyardgrass: flood tolerance, resistance to herbicides
  - Purple nutsedge: flood tolerance, lowland ecotype evolved

- We can learn from weeds
  - Crop improvement and productivity
    - Ability to adapt: Weed genetic diversity vs crop uniformity
  - Weed management with less herbicides or handweeding
    - "Weed-resistant" (competitive) crops
    - "Harmless" or self-destructive weeds

- "Weed resistance" and high-yielding traits in a single cultivar:
  is it possible?

World's worst weeds in 1977 and 24 years later (2001)

Species	Country	Crop	Species	CABI citation
C. rotundus	**92**	**52**	*C. dactylon (2)*	5000
C. dactylon	80	40	***E. crusgalli (4)***	**3000**
I. cylindrica	75	35	*S. halepense (7)*	2000
E. crusgalli	**61**	**36**	***C. rotundus (1)***	**2000**
E. colona	60	35	*D. ciliaris (21)*	1500
E. indica	60	36	*I. cylindrica (3)*	1000
S. halepense	53	30	*E. indica (6)*	1000
A. spinosus	54	28	*E. colona (5)*	1000
A. conyzoides	46	36	*R. cochinchinensis (17)*	500
D. aegyptium	45	19	*C. difformis (10)*	300
B. pilosa	40	31	*C. iria (19)*	300
E. prostrata	35	22	*P. conjugatum (16)*	200
R. cochinchinensis	28	18	*A. spinosus (8)*	200

Holm, Plucknett, Herberger and Pancho, 1977; Terry, 2001

A tale of two weeds: survival and adaptation mechanisms

“Superweeds”
weeds with widespread global distribution, which are difficult to control with conventional means

infests 52 crops
in 92 countries
one plant can produce
3 to 7 million tubers/ha

In the beginning, there was no barnyardgrass or purple nutsedge

Weeds in lowland rice in Muda area, Malaysia

TPR (1979)	DSR (1987)	WSR (1989)
***M. vaginalis* (b)**	***E. crusgalli* (g)**	***E. crusgalli* (g)**
L. hyssopifolia (b)	*E. colona* (g)	*L. chinensis* (g)
F. miliacea (s)	*L. chinensis* (g)	*F. miliacea* (s)
C. difformis (s)	*S. grossus* (s)	*M. crenata* (b)
L. flava (b)	*F. miliacea* (s)	*M. vaginalis* (b)

Weeds in lowland rice in Central Luzon, Philippines

TPR (1960)	DSR (1986)	DSR (2005)
***M. vaginalis* (b)(94%)**	***E. glabrescens* (g)**	***C. rotundus* (s)**
S. zeylanica (b)	***E. crusgalli* (g)**	***E. crusgalli* (g)**
L. octovalvis (b)	*F. miliacea* (s)	*S. zeylanica* (b)
C. iria (s)	*P. distichum* (g)	*I. rugosum* (g)
E. crusgalli (g) (1%)	*M. vaginalis* (b)	*P. distichum* (g)

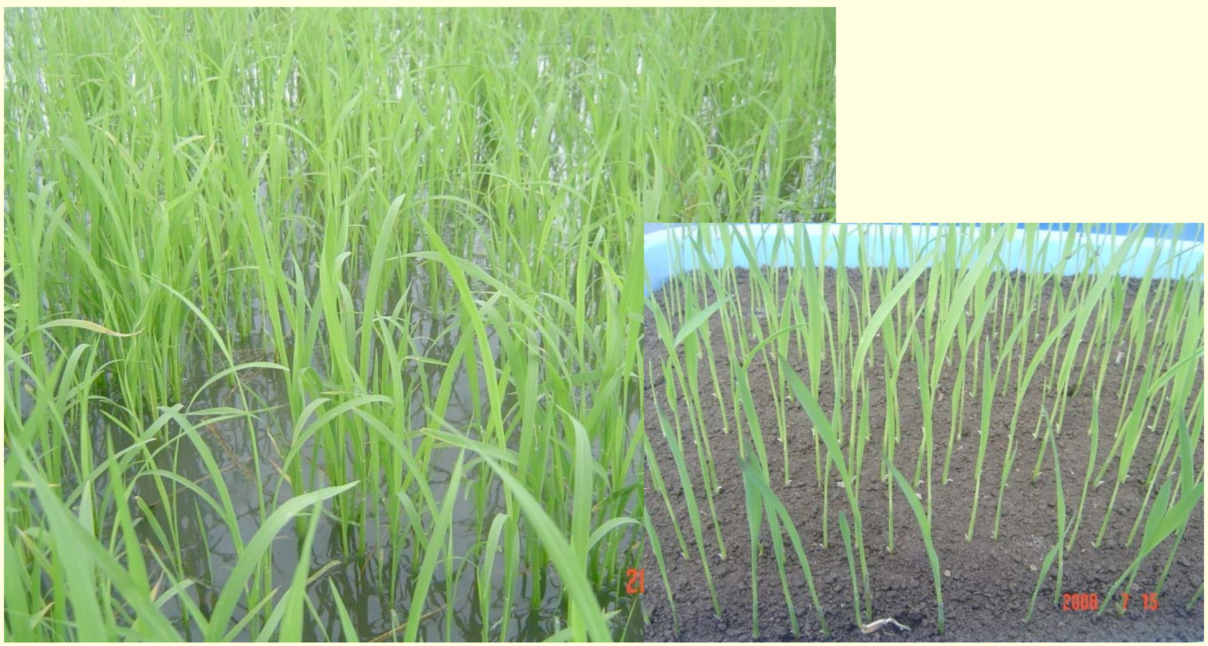
How did barnyardgrass replace broadleaves as major weed in rice?

Before 1970s

Transplanted rice

5-10 cm water

Dominant weeds: aquatic broadleaves and sedges



After 1970s

Direct-seeded rice:

Saturated soil enhances germination of both rice and grasses

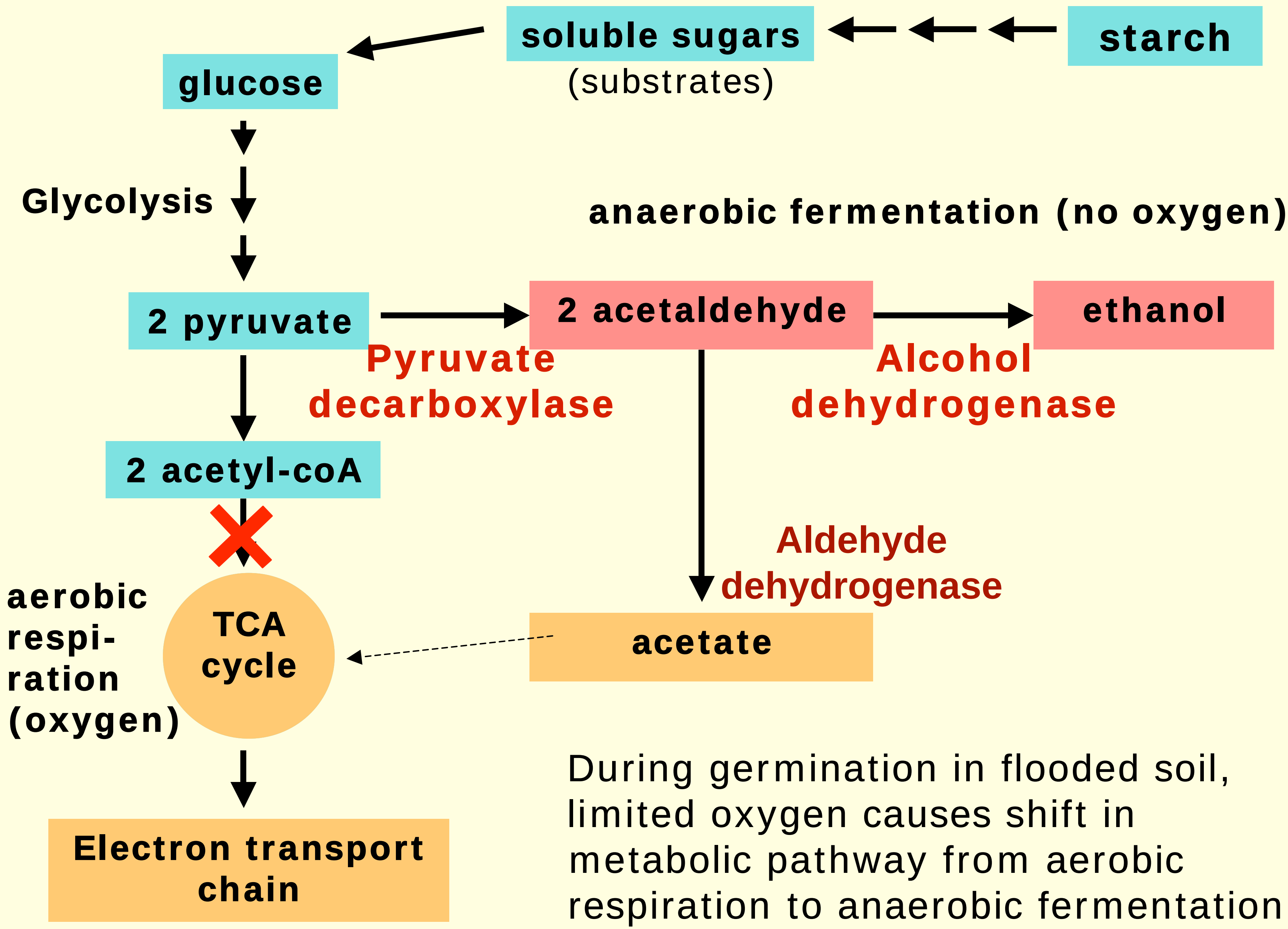
Dominant weeds shifted from broadleaves to grasses

Why does barnyardgrass thrive in flooded soil?

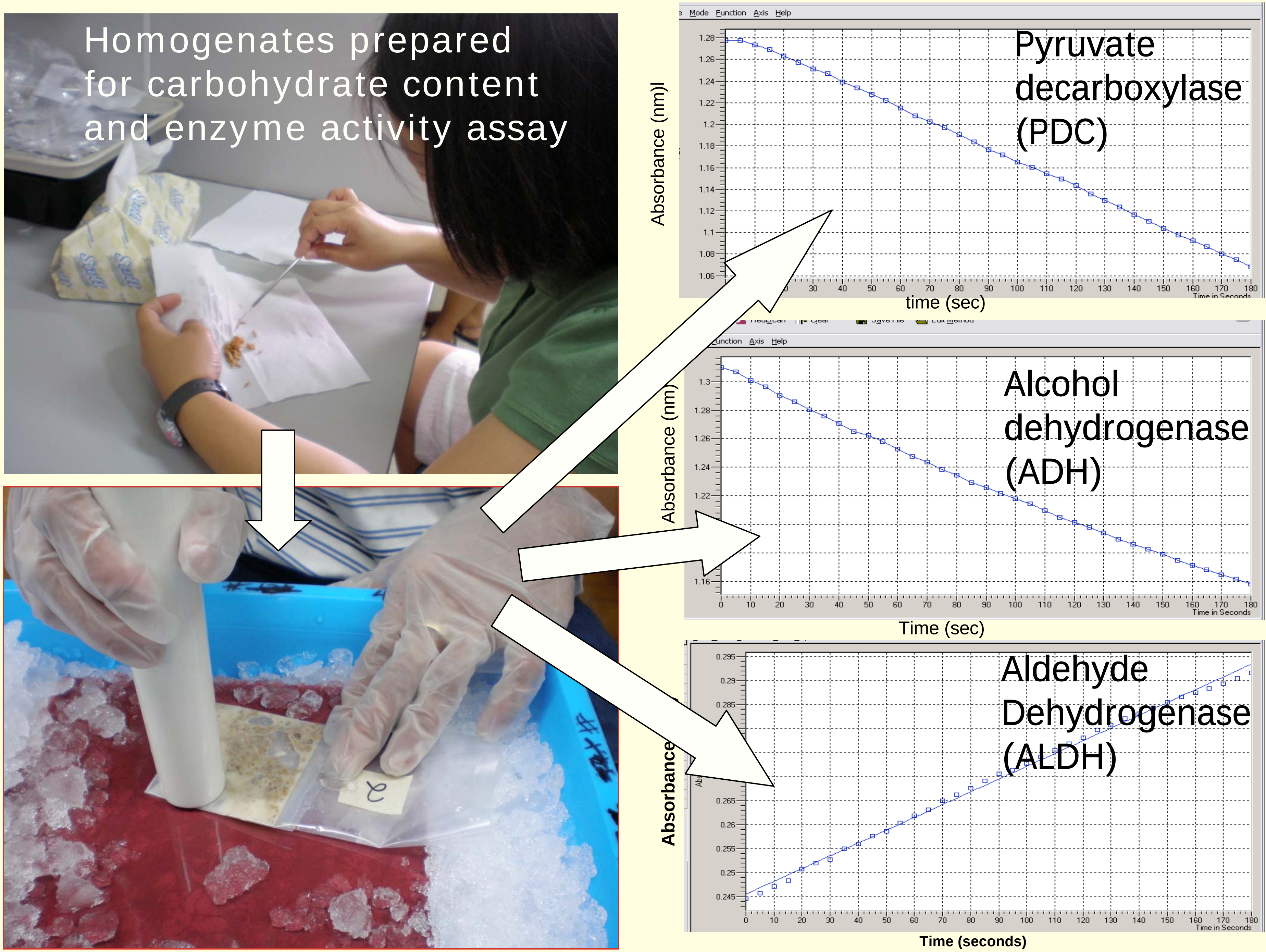
- We conducted studies to determine flood response mechanisms in rice and barnyardgrass
- We compared carbohydrate metabolism and anaerobic fermentation in germinating rice and barnyardgrass

Germinated in 5, 10, 20, 100 mm water applied at 0, 2, and 4 DAS

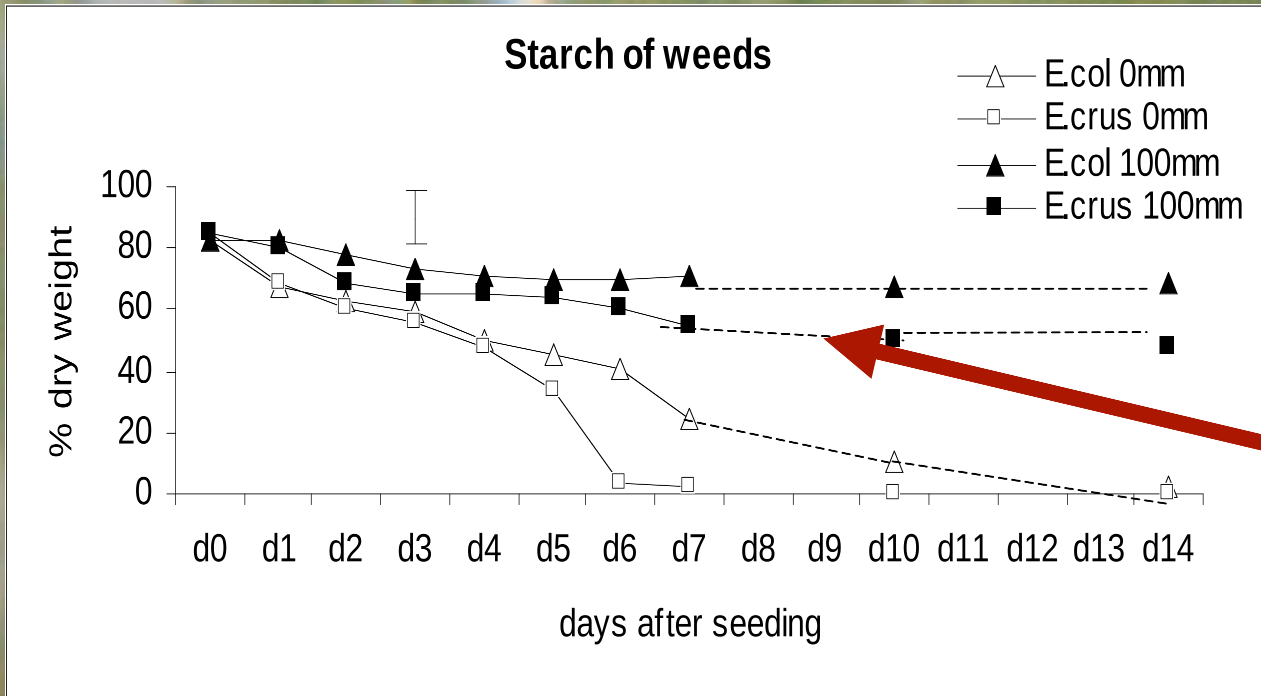
3 to 4 day-old seedlings were grown in 100mm

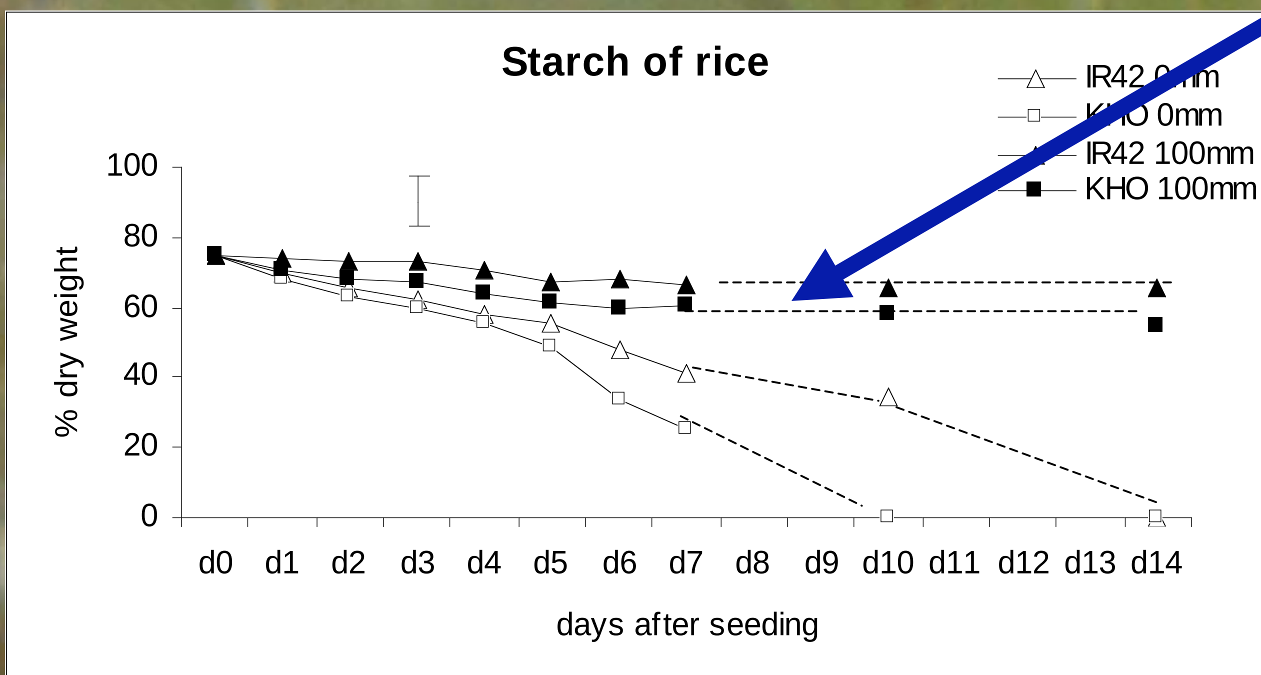


During germination in flooded soil, limited oxygen causes shift in metabolic pathway from aerobic respiration to anaerobic fermentation



Homogenates prepared
for carbohydrate content
and enzyme activity assay
Pyruvate
decarboxylase
(PDC)
Absorbance (nm)l
time (sec)
Alcohol
dehydrogenase
(ADH)
Absorbance (nm)
Time (sec)
Aldehyde
Dehydrogenase
(ALDH)
Absorbance
Time (seconds)



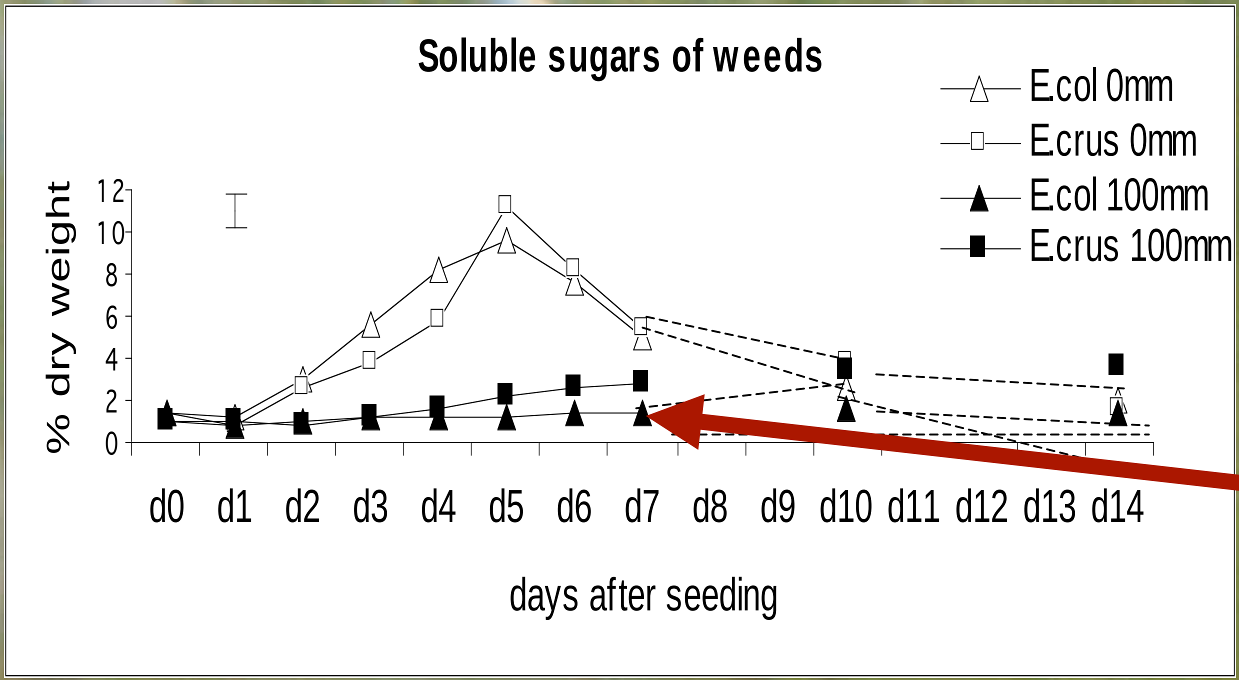


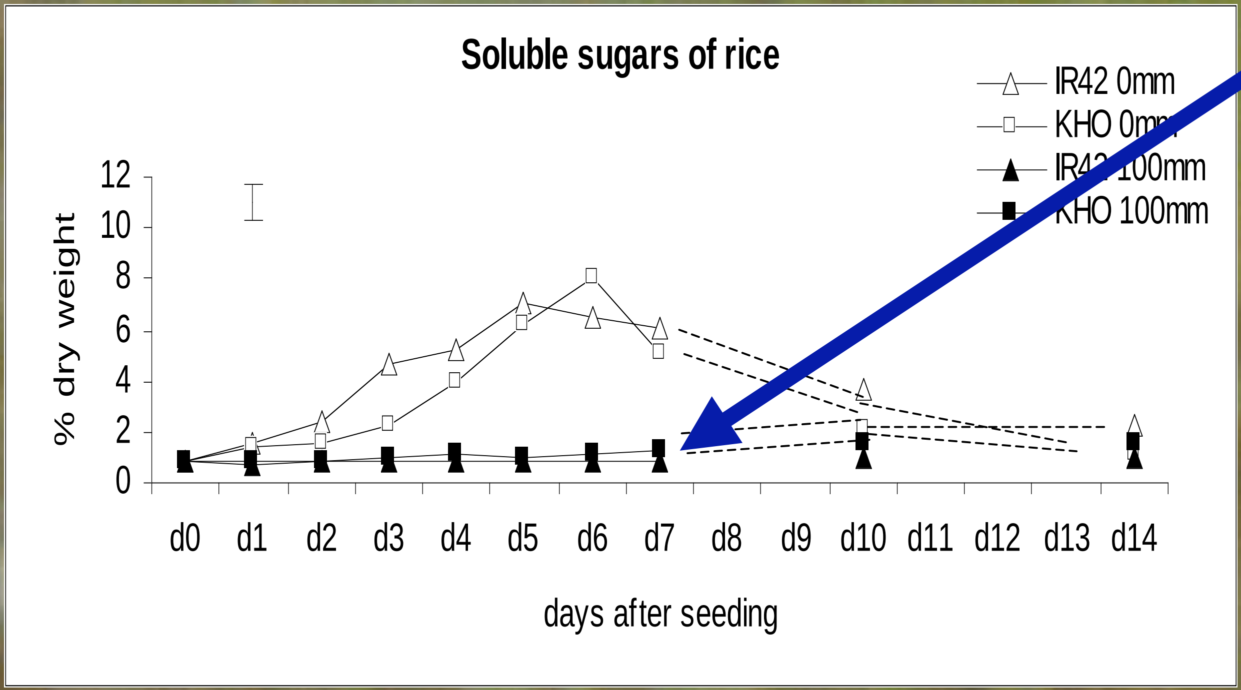
carbohydrate metabolism

Decreased ability of barnyardgrass and rice to degrade starch when flooded

Starch degraded to soluble sugars for anaerobic respiration

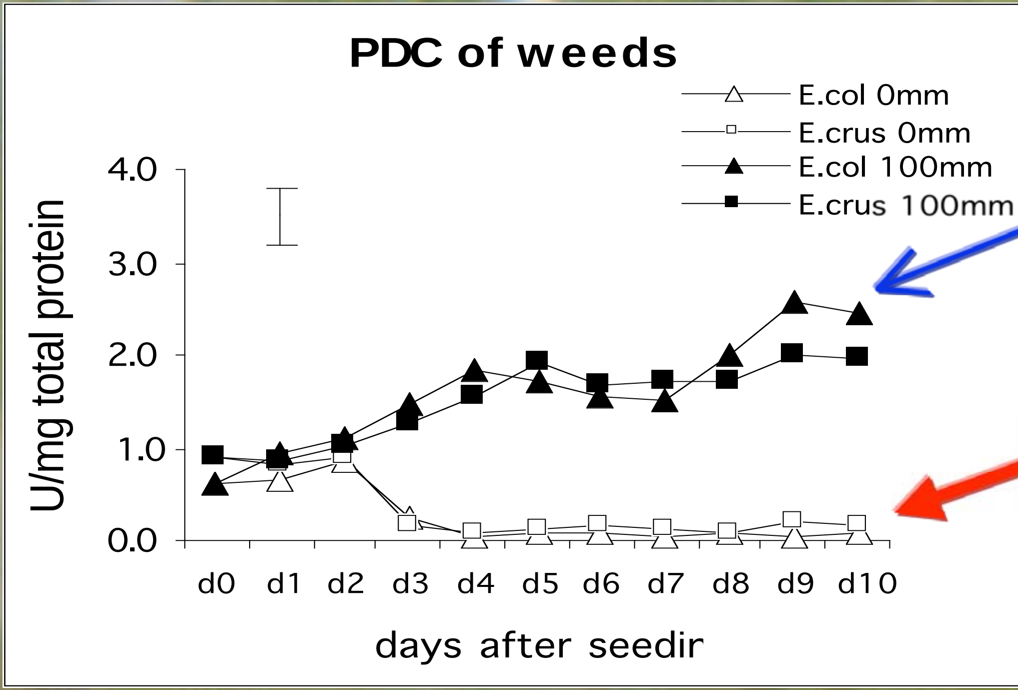
carbohydrate metabolism

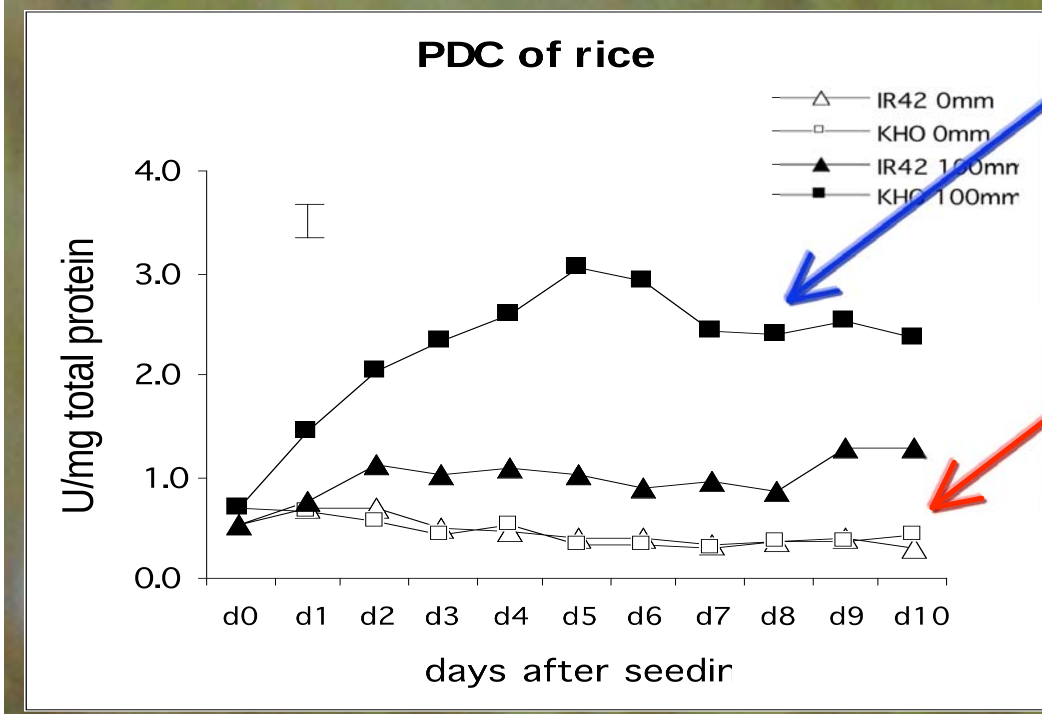




Decreased ability of barnyardgrass and rice to produce soluble sugars when flooded

Sugars are used as substrates in anaerobic fermentation





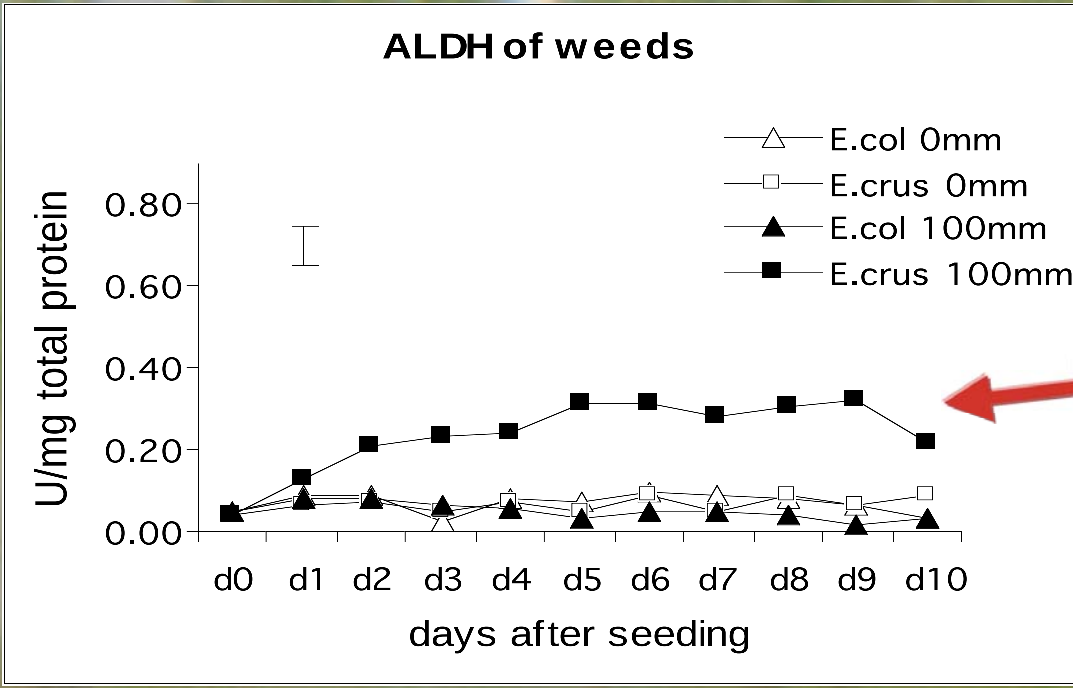
anaerobic fermentation

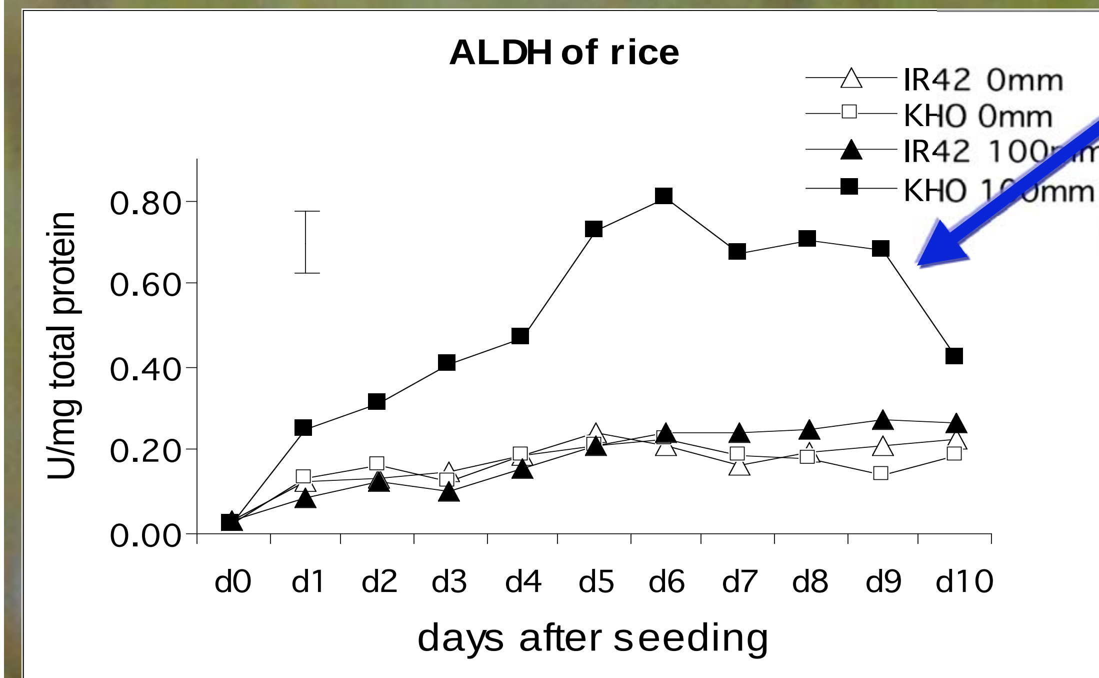
Barnyardgrass (flooded)
PDC and ADH increased
in anaerobic fermentation

Barnyardgrass (aerobic)
PDC and ADH shut down
No anaerobic fermerntation

Rice (flooded)
PDC and ADH increased
in anaerobic fermentation

Rice (aerobic)
PDC and ADH still active
both aerobic and anaerobic
processes taking place at
the same time





aldehyde dehydrogenase

ALDH activity in *E.crus-galli* much higher when submerged

ALDH activity in rice much higher when submerged

ALDH detoxifies acetaldehyde, which is toxic to plant cells, thus may play a role in flood tolerance of rice and barnyardgrass

Barnyardgrass eventually replaced broadleaves as major weeds in rice possibly because ….

- In flooded soil, barnyardgrass can undergo anaerobic fermentation just as well as rice.

- While rice undergoes some degree of aerobic respiration in flooded soil, barnyardgrass can shut down aerobic respiration completely in flooded soil.

- Barnyardgrass can easily recover fast from initial injury incurred in flooded soil.

germinating rice

germinating *E. crusgalli*

Fast recovery of *E.crusgalli* from flooding injury

Flooding depth	Shoot length (mm)		Root length (mm)	
	Barnyardgrass	Rice	Barnyardgrass	Rice
7 DAS				
0 mm	220	361	94	126
100 mm	194	361	74	118
% redn	12	0	22	6
14 DAS				
0 mm	274	386	237	162
100 m	371	483	144	144
% redn	0	0	39	11

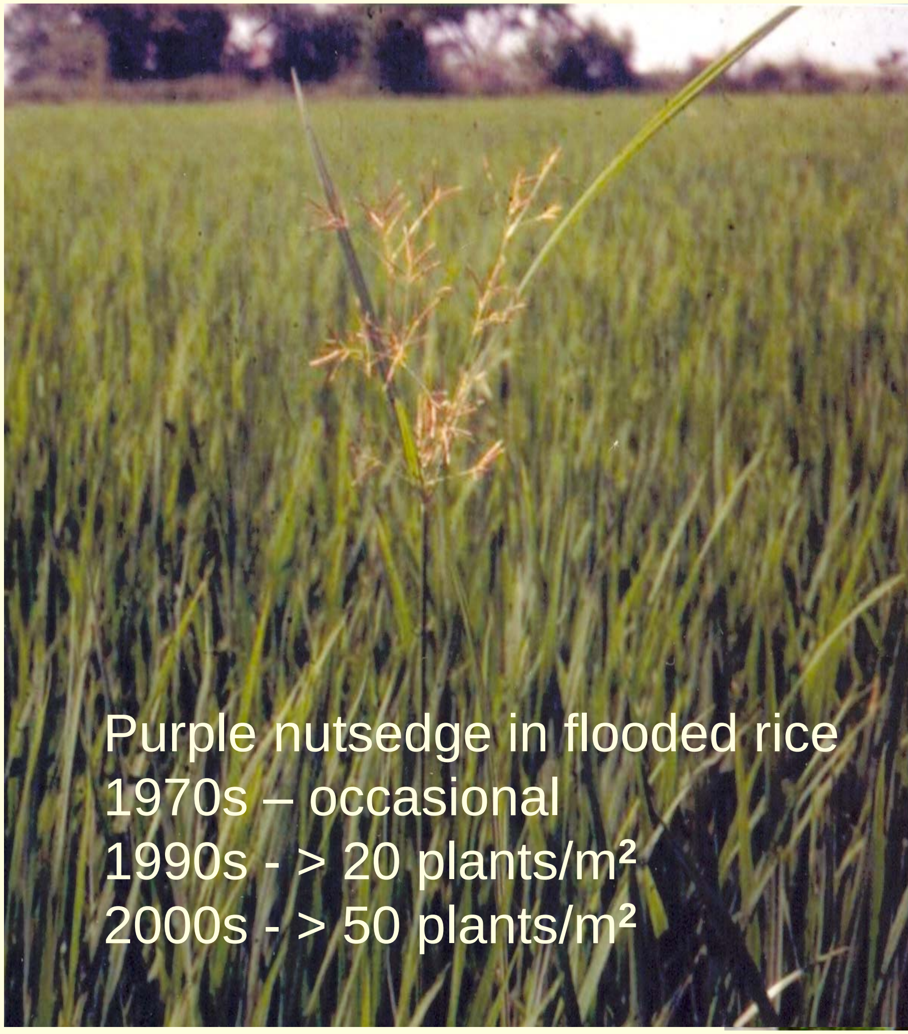
Purple nutsedge: A tough nut to crack

- 52 crops in 92 countries
- a single plant can produce 3 to 7 million tubers/ha
- tubers not controlled by herbicides
- farmers spend P10,000/ha for handweeding labor
- has evolved a lowland ecotype which has adapted to flooded soil

Continuous rice-vegetable rotation over the years is selecting for purple nutsedge that can grow in flooded soil

Tubers carried over into next crop, can cause weed population build-up

1996: Increasing occurrence of lowland purple nutsedge in lowland rice



Survey of weeds in rice-vegetable fields in Nueva Ecija from 1996 to 2000


Nueva Ecija
1998


Laguna
1998


Iloilo
2005


Panga
sinan
Pam
panga
Nueva
Ecija
Tarlac
Iloilo


Tarlac
2006


Bulacan
2006
07/17/2006

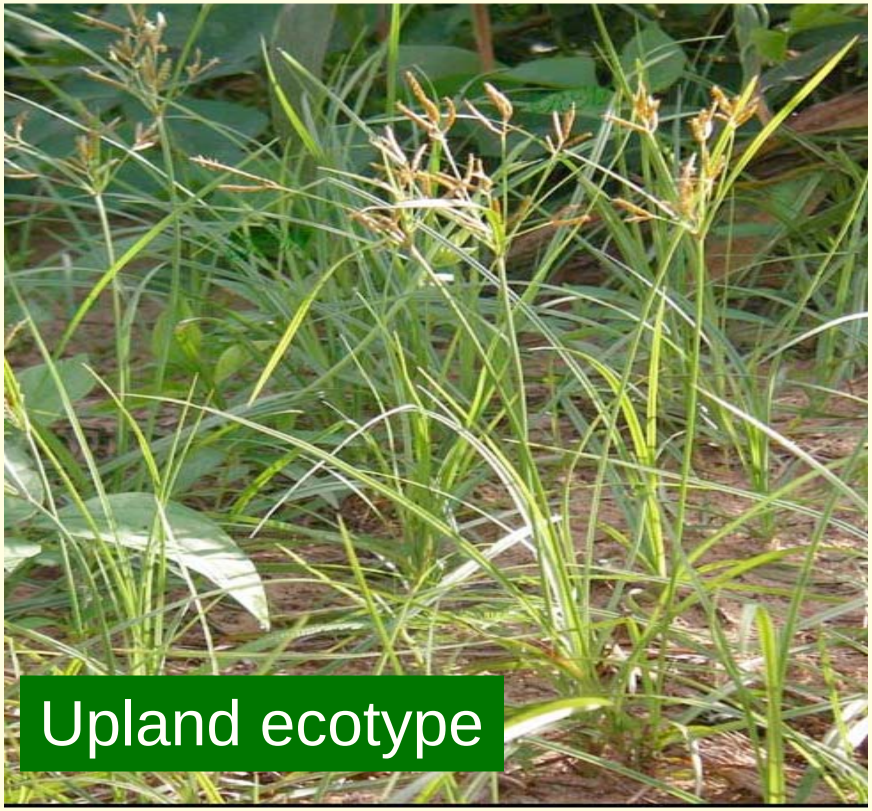

Pampanga
2006
07/17/2006

1996-2005: increase in lowland purple nutsedge populations

Weed species	Summed dominance ratio (2005)			
	Iloilo	N Ecija	Pangasinan	Tarlac
C. rotundus	81(6)*	74 (4)*	69 (4)*	87 (2)*
P. distichum	3	2	13	0
S. zeylanica	0.1	4	10	0
I. rugosum	12	1	1	0
H. zeylanica	0	13	4	0
E. crusgalli	2	1	1	0
C. difformis	0	1	3	6
F. miliacea	1	1	3	0
C. iria	0	1	0	0

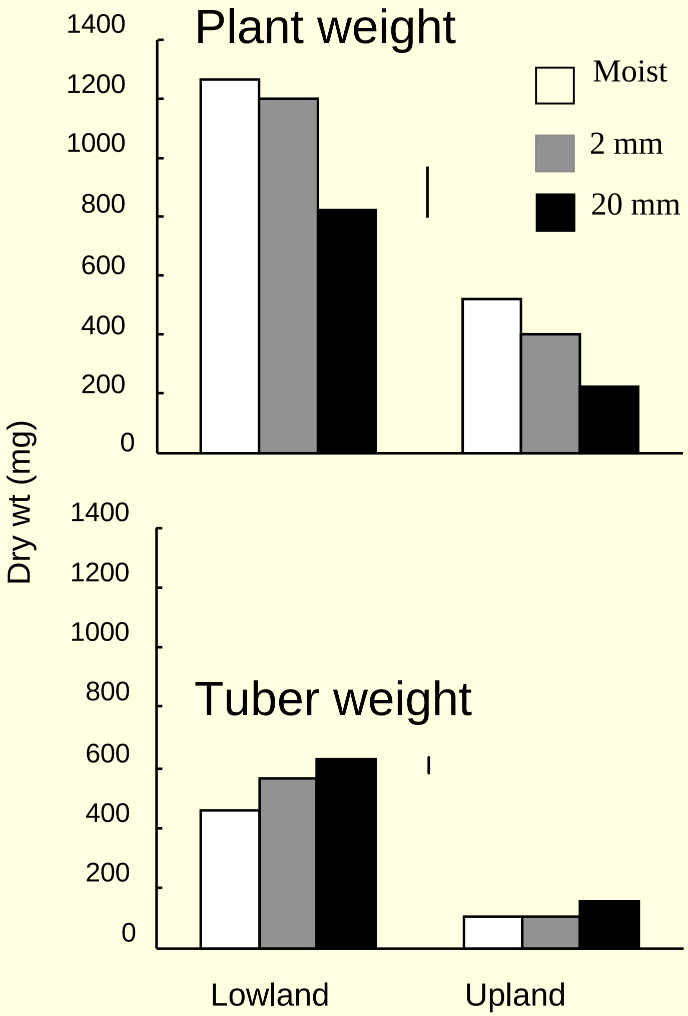
- Numbers in parenthesis: fields with 100% purple nutsedge populations
- Survey of 30 fields in 4 provinces, 2005 wet season

Appearance of lowland purple nutsedge means that a lowland ecotype has evolved a mechanism to adapt to flooded soil.



How did purple nutsedge adapt to flooded soil?
To answer this question, we compared morphological and biochemical features of upland and lowland ecotypes

Morphological comparison of upland and lowland ecotypes



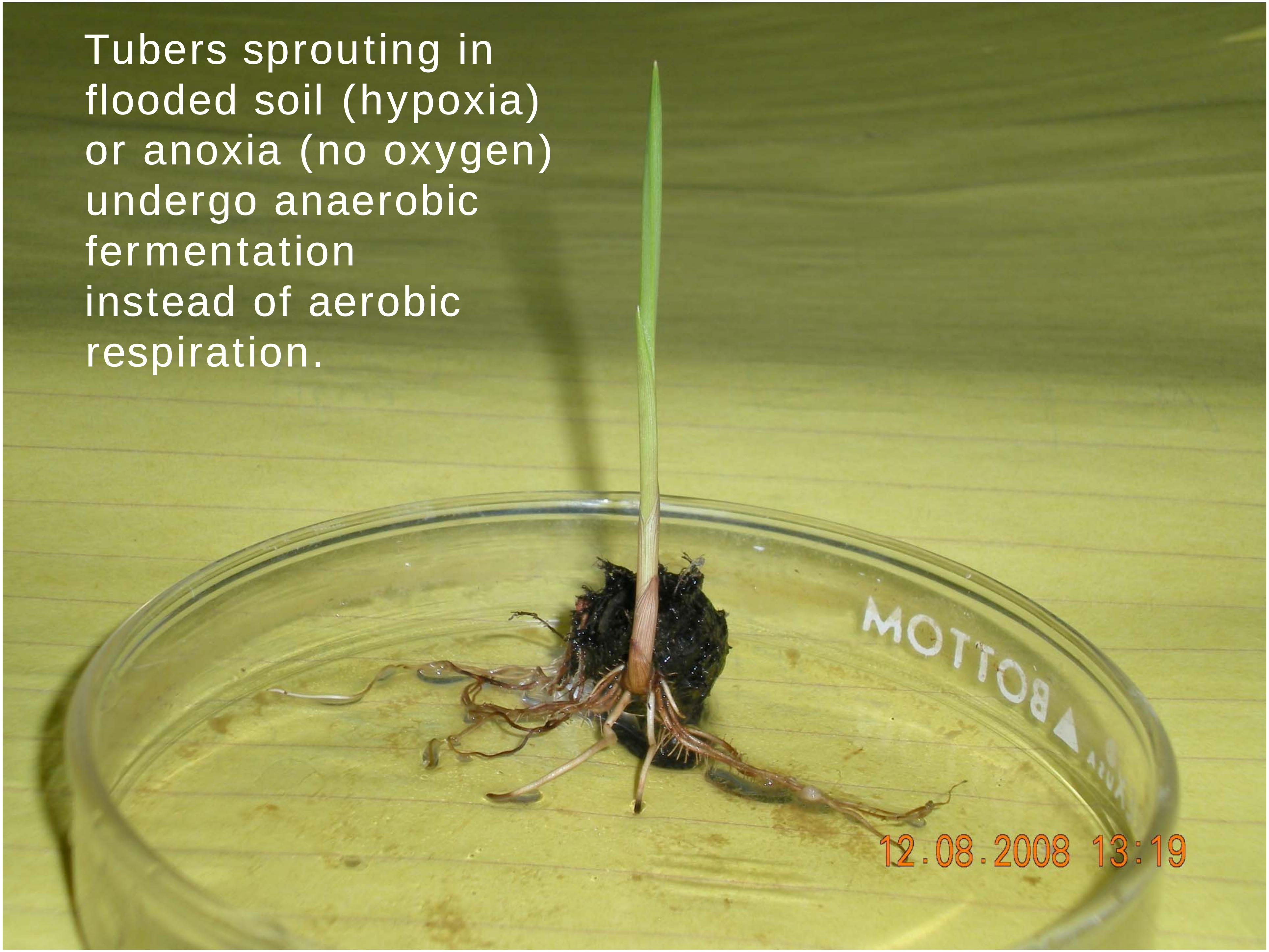
Lowland Upland

Air space in stem (aerenchyma)

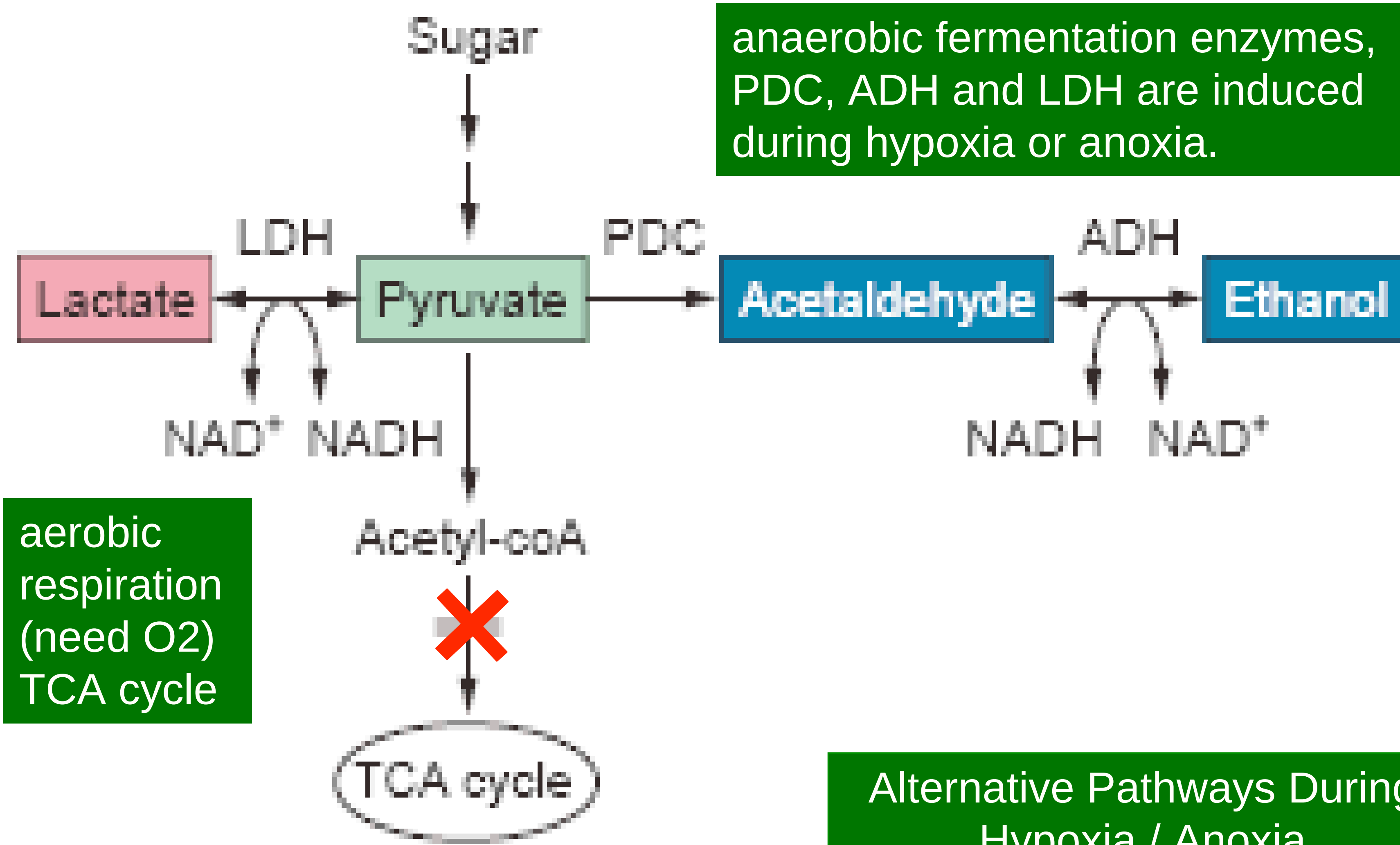
Wetland – 1.2 g

Dryland – 0.4 g

Tubers sprouting in flooded soil (hypoxia) or anoxia (no oxygen) undergo anaerobic fermentation instead of aerobic respiration.



How did lowland purple nutsedge adapt to flooded soil?

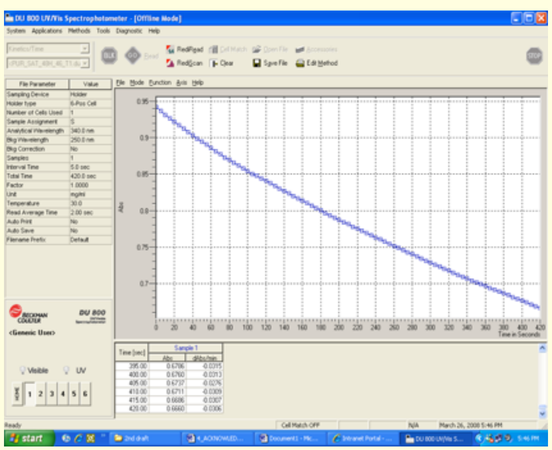


anaerobic fermentation enzymes, PDC, ADH and LDH are induced during hypoxia or anoxia.

aerobic respiration (need O2) TCA cycle

Alternative Pathways During Hypoxia / Anoxia

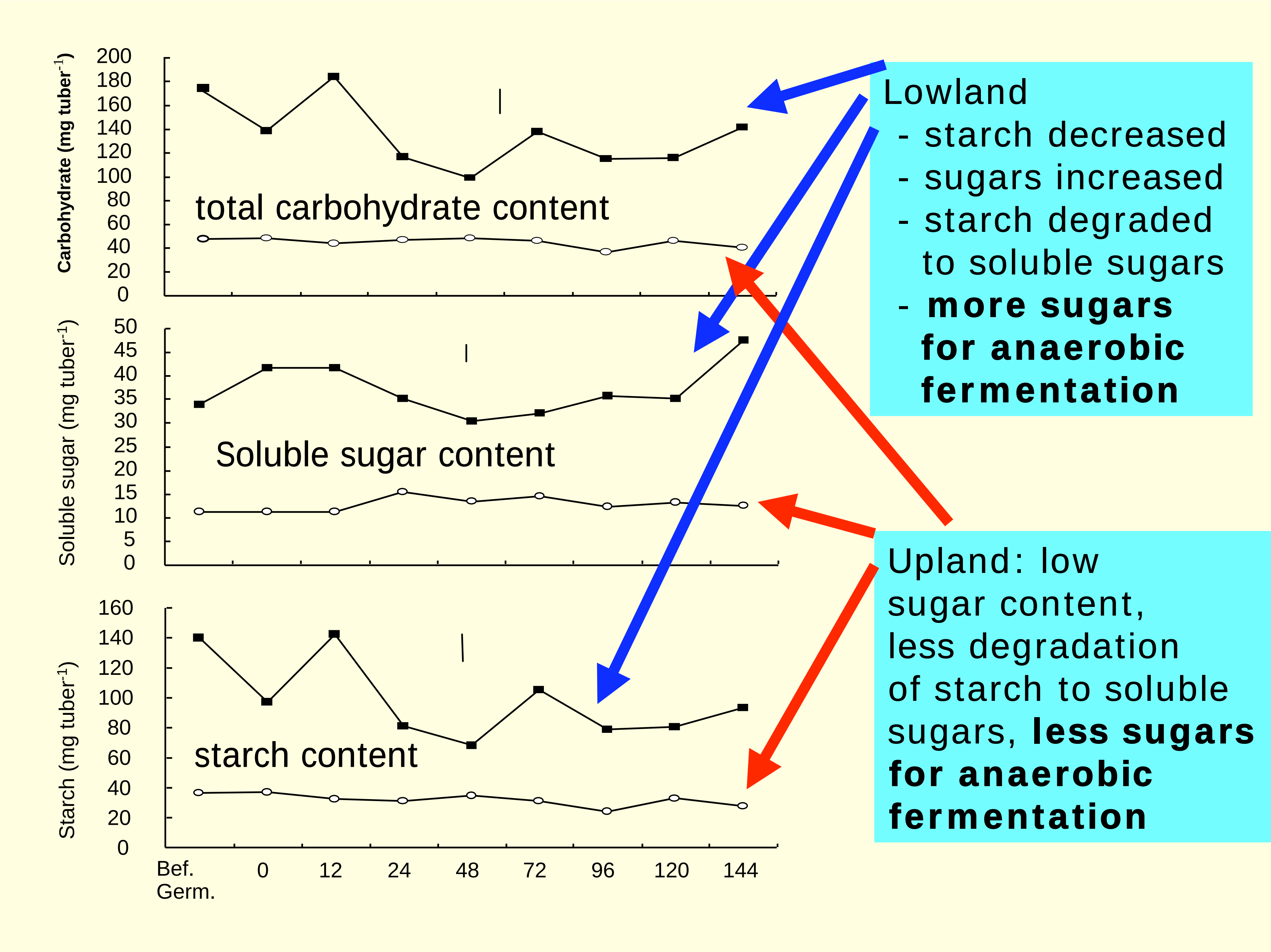
Carbohydrate content and enzyme activity assayed every 24 hrs for 6 days



Carbohydrate, starch, soluble sugar content in tubers

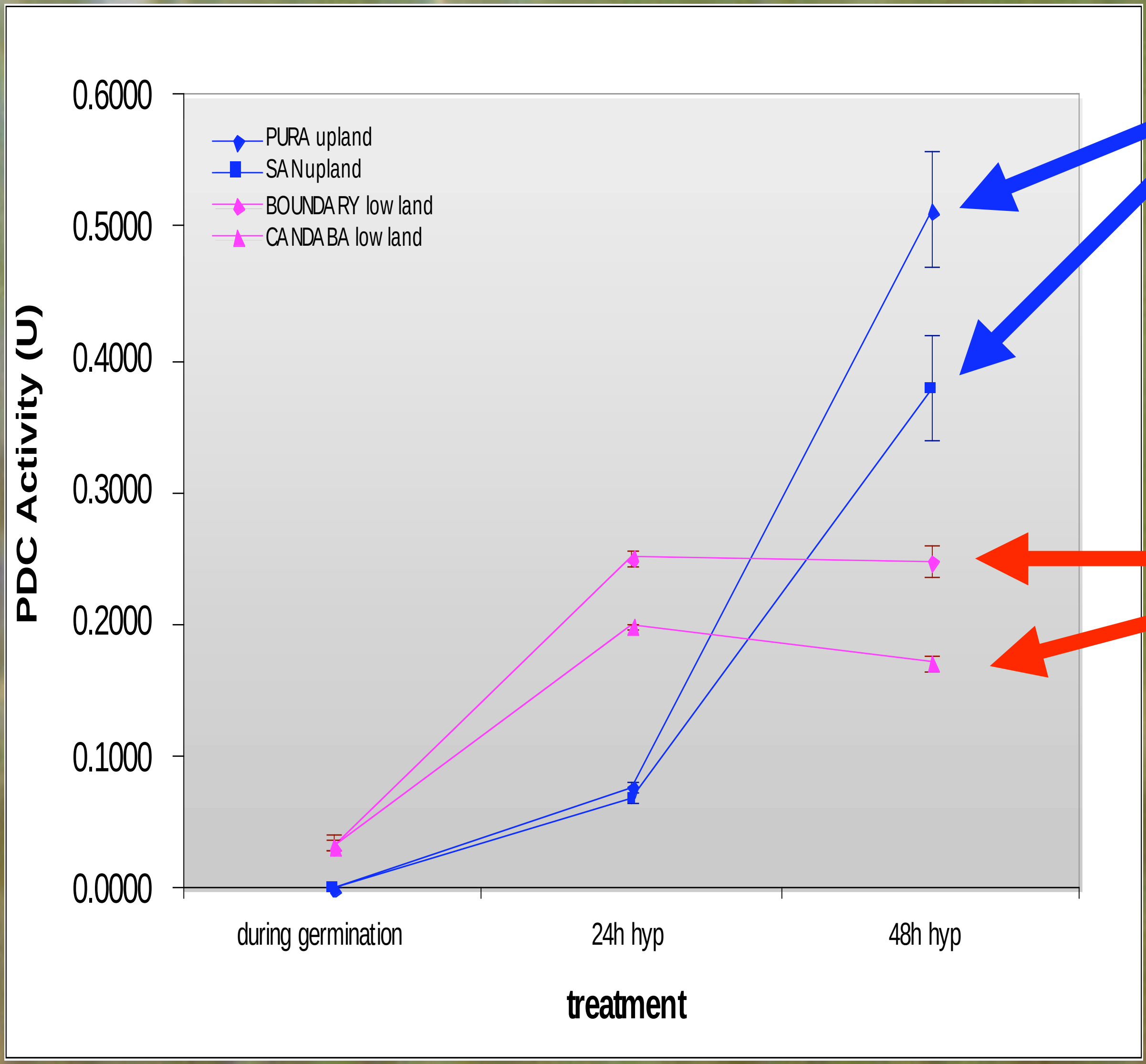
Enzyme activity in roots

- Alcohol dehydrogenase (ADH)
- Pyruvate decarboxylase (PDC)
- Lactate dehydrogenase (LDH)



Carbohydrate (mg tuber-1)
200
180
160
140
120
100
80
60
40
20
0
total carbohydrate content
Soluble sugar (mg tuber-1)
50
45
40
35
30
25
20
15
10
5
0
Soluble sugar content
Starch (mg tuber-1)
160
140
120
100
80
60
40
20
0
starch content
Bef. Germ.
0
12
24
48
72
96
120
144
Lowland
- starch decreased
- sugars increased
- starch degraded to soluble sugars
- more sugars for anaerobic fermentation
Upland: low sugar content, less degradation of starch to soluble sugars, less sugars for anaerobic fermentation

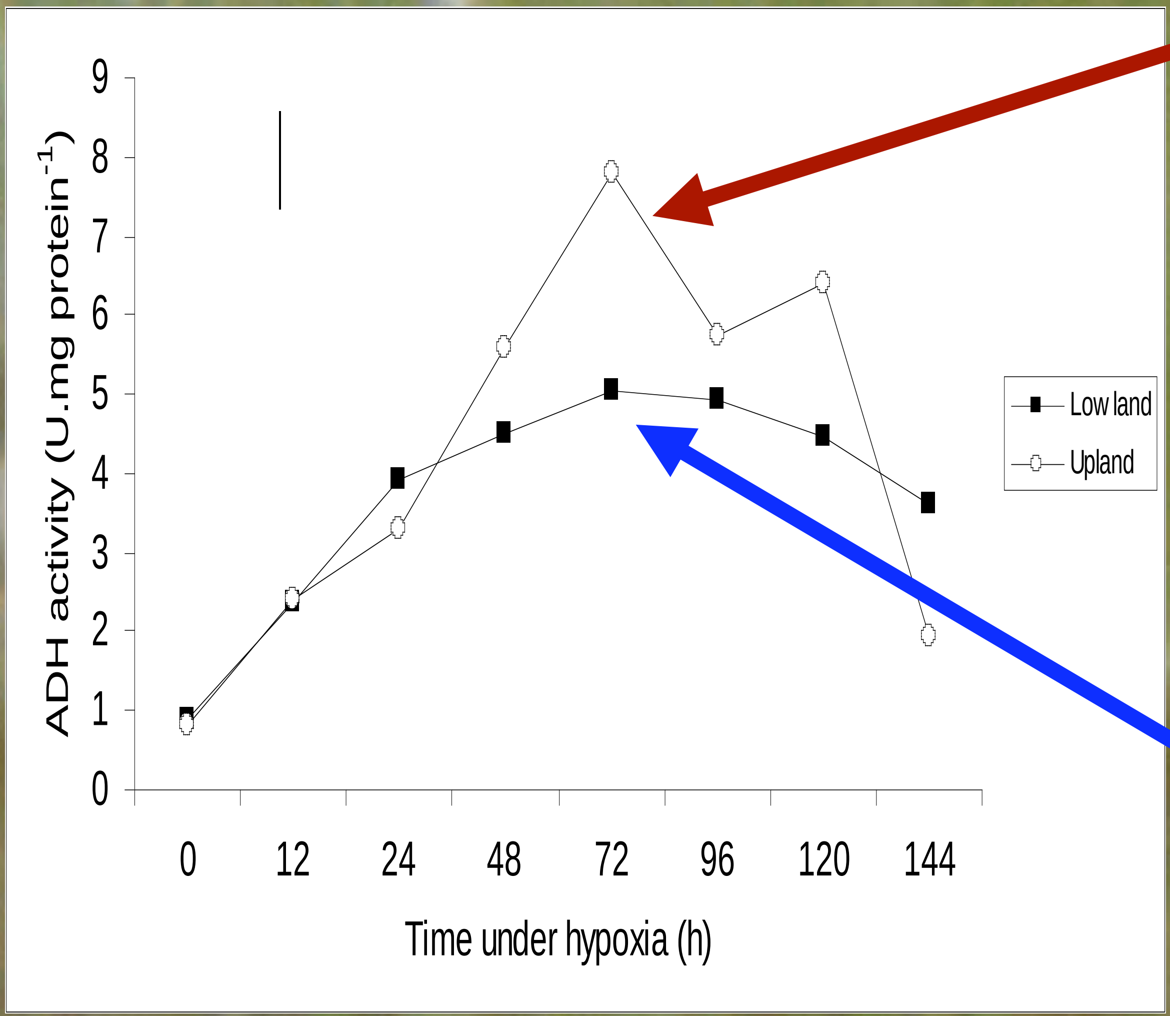
Pyruvate decarboxylase activity in ecotypes



Upland: continued fast increase of enzyme activity until sugars are depleted

Lowland: slow but constant down-regulated activity to conserve sugars and sustain anaerobic fermentation

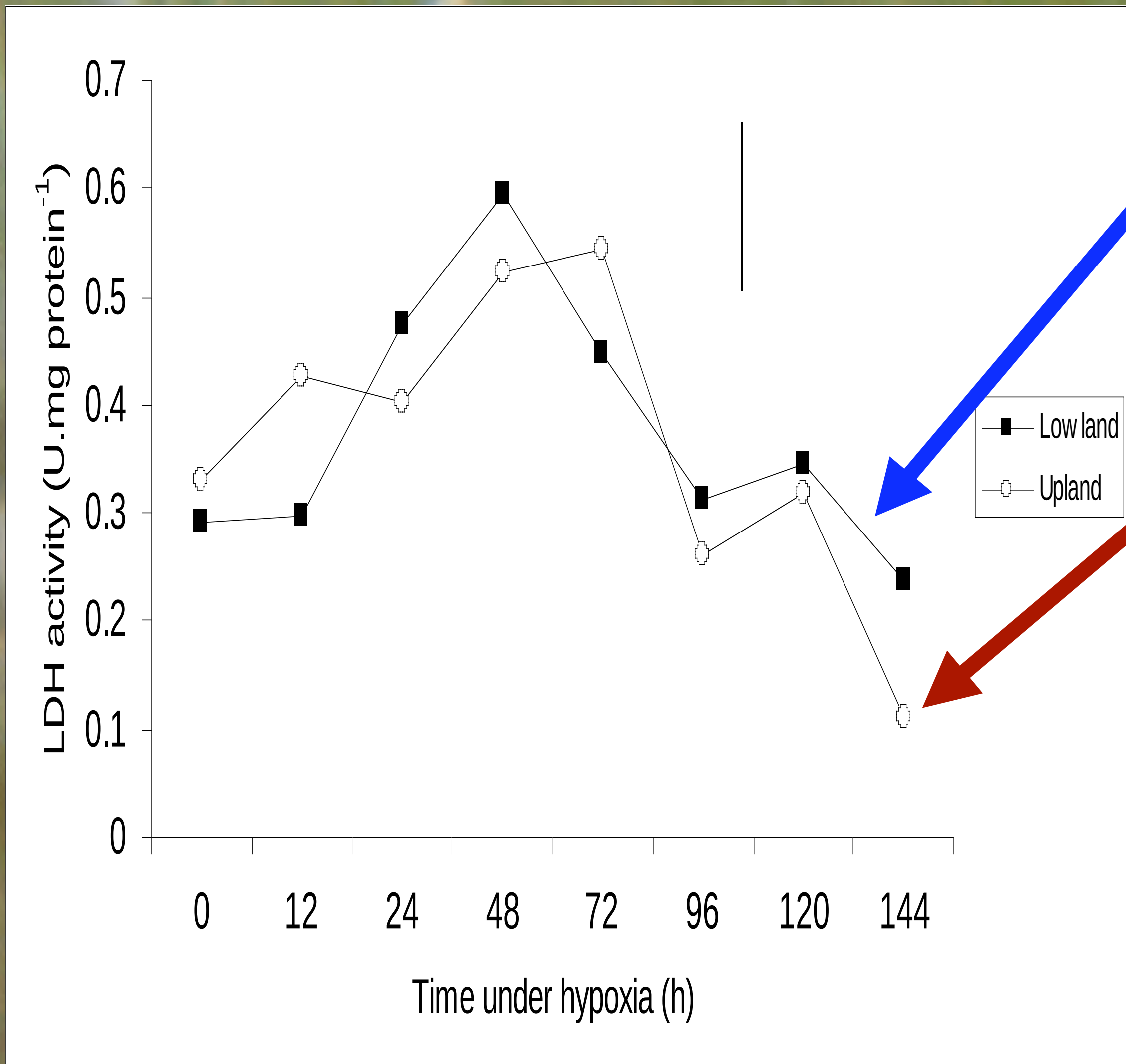
Alcohol dehydrogenase activity in ecotypes



Upland**:** sharp increase then abrupt decrease **due to sugar depletion**

Lowland**:** slow increase and constant down-regulated activity **to conserve sugars and sustain anaerobic fermentation**

Lactate dehydrogenase activity in ecotypes



Lowland: higher LDH activity than upland after 6 days

Prevent lactic acid accumulation and acidic pH in cytoplasm in lowland ecotype

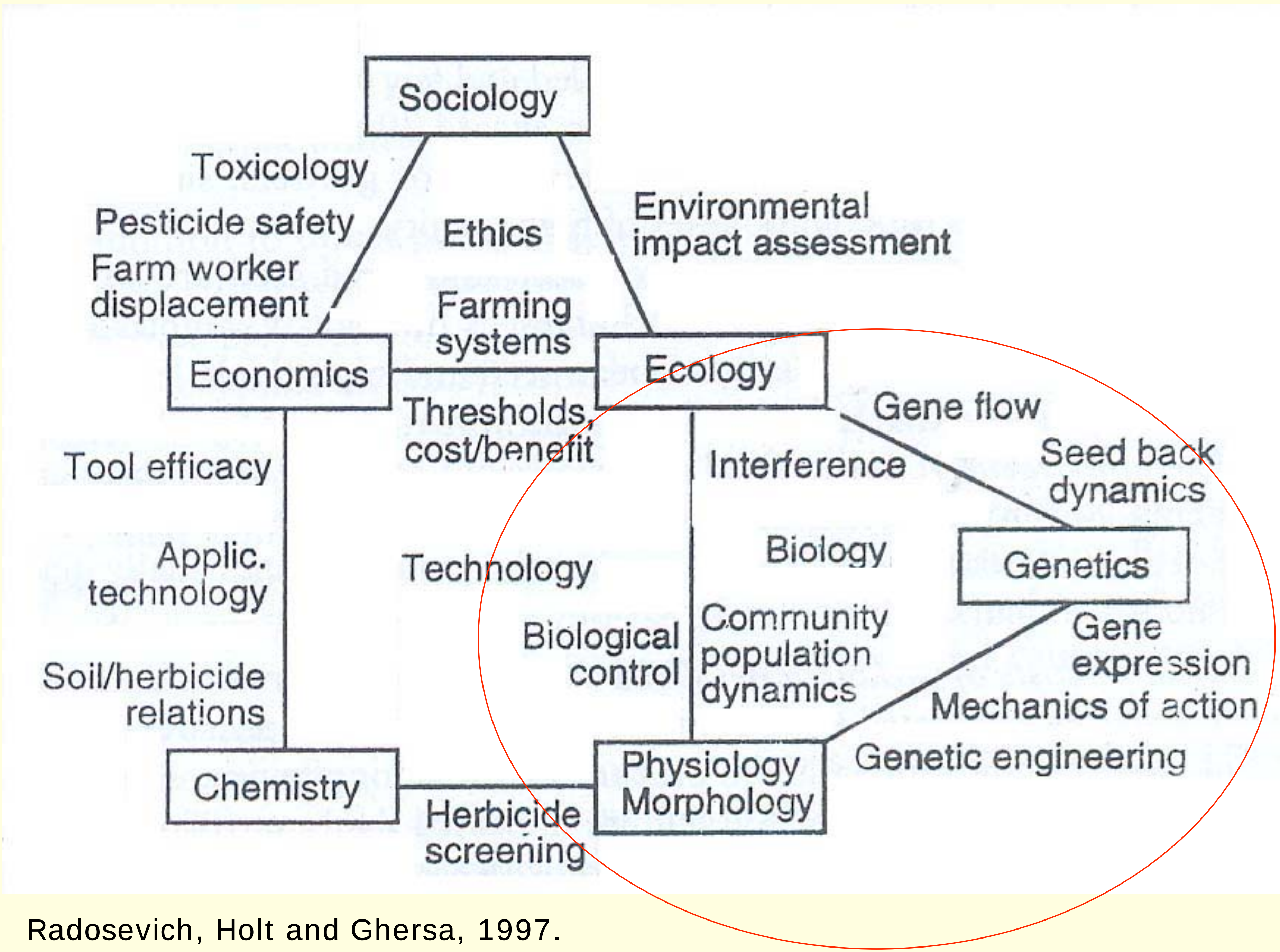
Lowland purple nutsedge flood tolerance mechanisms

Morphological

- Taller plants, bigger tubers than upland ecotype
- More carbohydrate, starch and soluble sugar content in tubers
- More aerenchyma (air spaces) in roots and stems to diffuse oxygen into submerged parts

Physiological

- More starch breakdown into soluble sugars
- Down-regulates PDC and ADH to conserve sugars to sustain anaerobic fermentation probably until its first leaf can undergo aerobic respiration
- Higher LDH activity prevents lactic acid accumulation and acidic pH in cytoplasm



Radosevich, Holt and Ghersa, 1997.
Weed Ecology: Implications for Management

We can learn from weeds

Crop improvement and productivity

Lessons from weed adaptation mechanisms

- Ability to adapt to environment (weed plasticity) is related to genetic diversity
- Weeds – high genetic diversity and extremely variable at the enzyme level
- Crops – genetic uniformity for high yields

- Genus *Echinochloa* has 48 species, including subspecies and varieties
- Each variety has different traits and different responses to environment or control methods

We can learn from weeds

Crop improvement and productivity

Lessons leaned from weed adaptation mechanisms

Compare crop genomes and weed genomes

- Only few researches are devoted to study of weed genomes
- Weeds are rich sources of genes which could be used to improve crop adaptation to adverse environment

Most crops are C3 while most weeds are C4

C3 – less competitive
- less efficient in photosynthesis

C4 – more competitive
- more efficient in photosynthesis

We can learn from weeds

Crop improvement and productivity
Lessons learned from weed adaptation mechanisms

- Identification of flood tolerant enzymes in weeds can pave the way for identification of genes that confer flood tolerance

- Incorporate flood-tolerant traits into flood-susceptible crops such as certain rice cultivars and other flood-susceptible crops

We can learn from weeds

Crop improvement and productivity

Lessons learned from weed adaptation mechanisms

- Silencing of genes controlling ADH and PDC in rice for greater competitiveness (rice undergoes both aerobic and anaerobic fermentation in aerobic soil)

- Incorporate genes to enhance LDH and ALDH in flood - susceptible crops to prevent acidosis in flooded soil

- Silencing of genes controlling LDH and ALDH in flood-tolerant weeds to make them flood-susceptible

We can learn from weeds

Weed management – search for sustainable,
innovative strategies

Managing weeds with less chemicals
and direct removal inputs like handweeding

- Modify crops to enhance competitiveness against weeds
  "Weed-resistant" crops
  - Develop allelopathic crops, C4 crops

- Modify weeds to reduce competitiveness against crops
  "Harmless" or "self-destructive" weed
  - convert weeds to being innocuous wild species
    (wild weed species are less competitive)

(Gressel, J. 2002. Molecular biology of weed control)

Existing management strategies

Chemical control: Development of herbicide-resistant weeds

Manual control: back-breaking work, labor costs constantly increasing

Need for innovative strategies that enhance crop competitiveness and/or reduce weed competitiveness to reduce direct removal inputs

Herbicides for control of barnyardgrass and herbicide-resistant barnyardgrass

Yr	Herbicide	Resistant species	when	Yrs	Resistance mechanism
1960	Propanil	*E. crusgalli*	1989	29	degradation
1969	Thiobencarb	*E. crusgalli*	1993	28	
1970	Butachlor	*E. crusgalli*	1993	23	
1970	Molinate	*E. crusgalli*	2000	30	
1974	Pendimethalin				
1983	Sethoxydim				
1987	Mefenacet				
1988	Pretilachlor	*E. crusgalli*	2004	16	
1989	Fenoxaprop	*E. crusgalli*	2000	11	insensitive
1989	Quinclorac	*E. crusgalli*	1998	9	target site
1993	Pyributicarb				
1995	Cyhalofop	*E. crusgalli*	2000	5	insensitive
1995	Flufenacet				target site
1997	Fentrazamide				
1997	Bispyribac	*E. phyllopogon*	2000	3	
2000	Metamifop				
2003	Flucetosulfuron				
2004	Penoxsulam				
2007	Pyribenxozim				

Managing weeds with less chemicals

Modify crops to increase weed competitiveness

Search for and develop allelopathic rice cultivars

Allelopathic crop – secretes chemicals which will kill, injure or inhibit growth of sorrounding plants (weeds)

Screening for allelopathic rice cultivars being done in various weed research labs in U.S. and Asia

Allelochemicals being identified and isolated

Identify genes responsible for production of allelochemicals; introduce allelopathy genes into crops

Modify crops to increase competitiveness

Develop a C4 rice cultivar

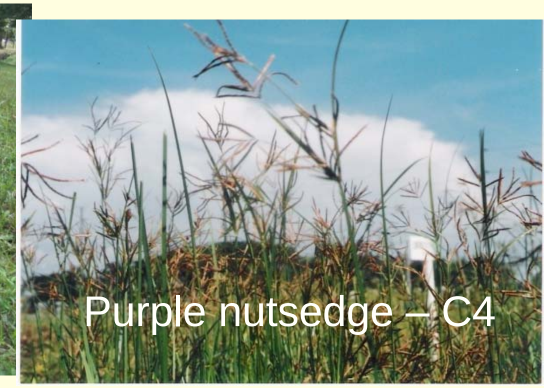
Plants produce sugars in photosynthesis thru C3 or C4 pathway

C4 more efficient, high-yielding, more competitive than C3 plants

Most tropical grass weeds are C4 plants, rice is a C3 plant

IRRI research: Develop C4 rice (Leung et al 2008)

- Compare morphology and physiology of rice with C4 plants (wild rice, C4 weeds)
- Compare C4 weed genomes with rice genome, identify genes coding for C4 pathway



Managing weeds with less chemicals

Modify weeds to reduce competitivenes

Develop “self-destructive” or “harmless” weeds

- Incorporate “self-destructive” genes into weeds
  - genes that inhibit growth
  - genes that mimic herbicide action
  - genes that modulate hormone levels

(Gressel, J. 2002. Molecular biology of weed control)

Managing weeds with less chemicals

Modify weeds to reduce competitivenes

Develop “self-destructive” or “harmless” weeds

Genes as target sites for herbicide action

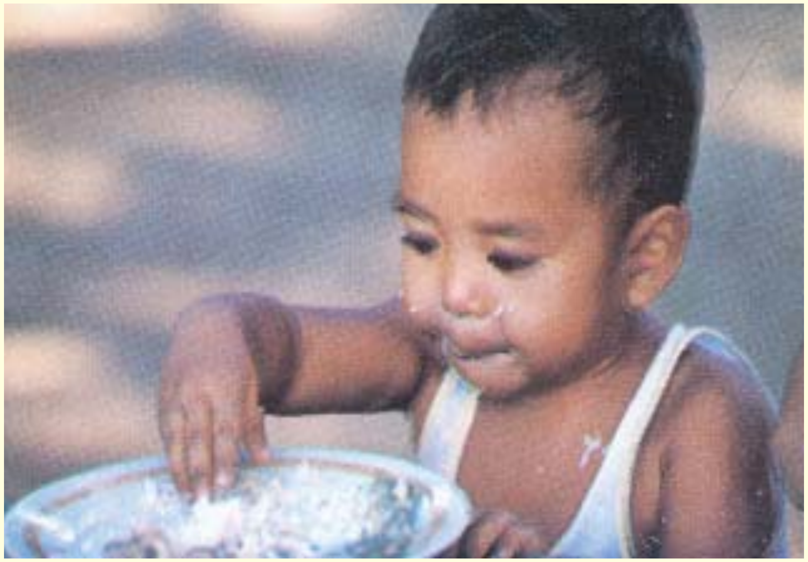
- Silencing genes for ALDH and LDH in weeds to enhance weed susceptibility to flooding

- Silencing genes that modulate ADH and PDC activities in weeds to enhance susceptibility to flooding

However....Why wont plant breeders do what weed scientists ask?

Because ....

- genes that confer competitiveness are in contrast to those that confer high yields – vegetative vs reproductive growth
- thus, plant breeders opt for high-yielding traits (but need maximum weed control inputs)
- need closer and wider collaboration among weed scientists, geneticists, plant breeders, agronomists and plant physiologists
- weed competitiveness and high-yielding traits in a single cultivar – is it possible?



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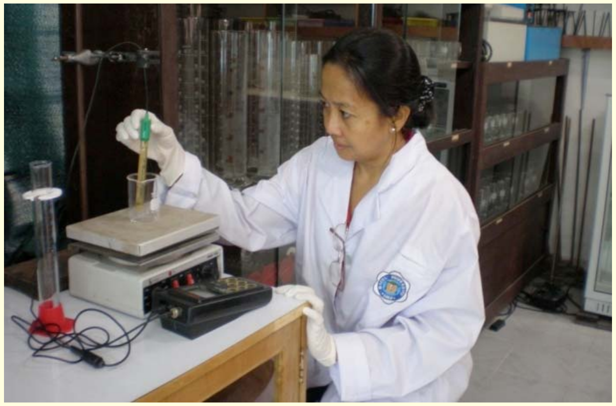

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