HERBICIDE-RESISTANT SOYBEAN AND CORN AS WEED MANAGEMENT TOOLS: BENEFITS AND RISKS FROM A FARMER'S PERSPECTIVE

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Weeds

- Weed pose a recurrent and ubiquitous threat to profitable crop production
- Weed control methods
  - Hand weeding - 10,000 B.C.
  - Primitive hand tools – 6,000 B.C.
  - Animal powered implements – 1000 B.C.
  - Row crop cultivation with animal power – 1731 A.D.
  - Mechanical powered implements – 1920
  - Biological control – 1930
  - Chemical weed control – 1947
  - Herbicide-resistant crops - 1993

Herbicides - the dominant method of weed control in the USA

Herbicide use and cost in corn and soybean, 2001

	Area treated		Cost $/year	
Crop	%	ha (million)	%	$ (billion)
Corn	98	30.0	35	2.3
Soybean	96	28.8	32	2.1

Note: Herbicide cost includes application and technology fees. Total herbicide cost for USA was 6.6 billion dollars.

Gianessi et al. 2002; www.ncfap.org

Herbicides

- Non-selective herbicides have limited utility - crop injury
  - e.g., glyphosate, glufosinate, paraquat.
- Traditionally, herbicides have been tailored for crops rather than the crops being bred to tolerate herbicides
- During the past decade, advances in biotechnology coupled with plant breeding have led to the development of herbicide-resistant crops.
  - Crops are being bred to tolerate existing herbicides.

Herbicide-resistant crops (HRCs)

- HRCs as a weed management tool is an extension of chemical weed control.
  - HRCs survive herbicide treatment that previously would have killed.

- There are two types of HRCs.

  1. **Transgenic HRCs:** Created through incorporation of foreign genes coding for specific traits.

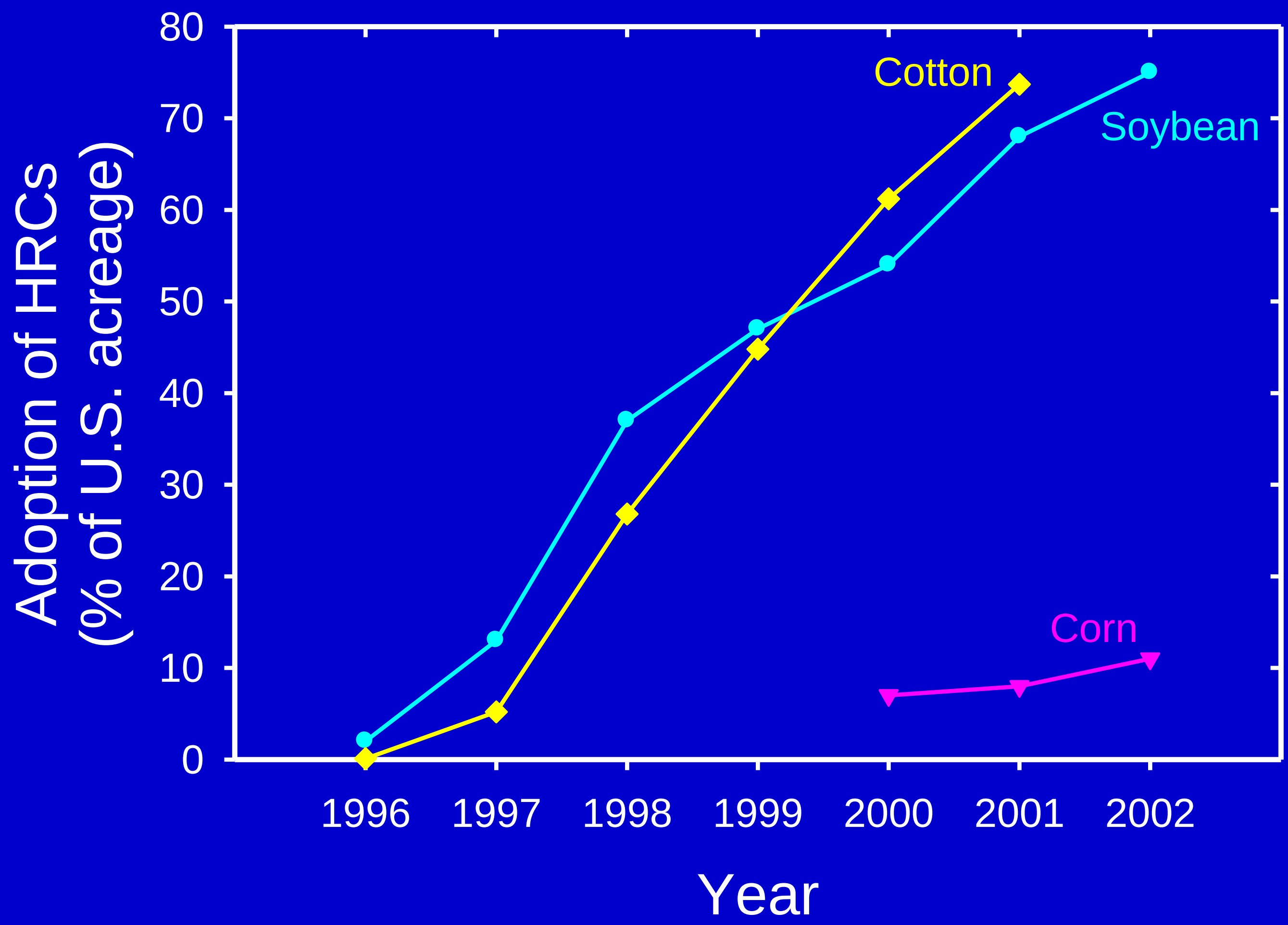
     e.g., Roundup Ready soybean
     BXN cotton

  2. **Non-transgenic HRCs:** Developed through traditional plant breeding using herbicide-resistant relatives.

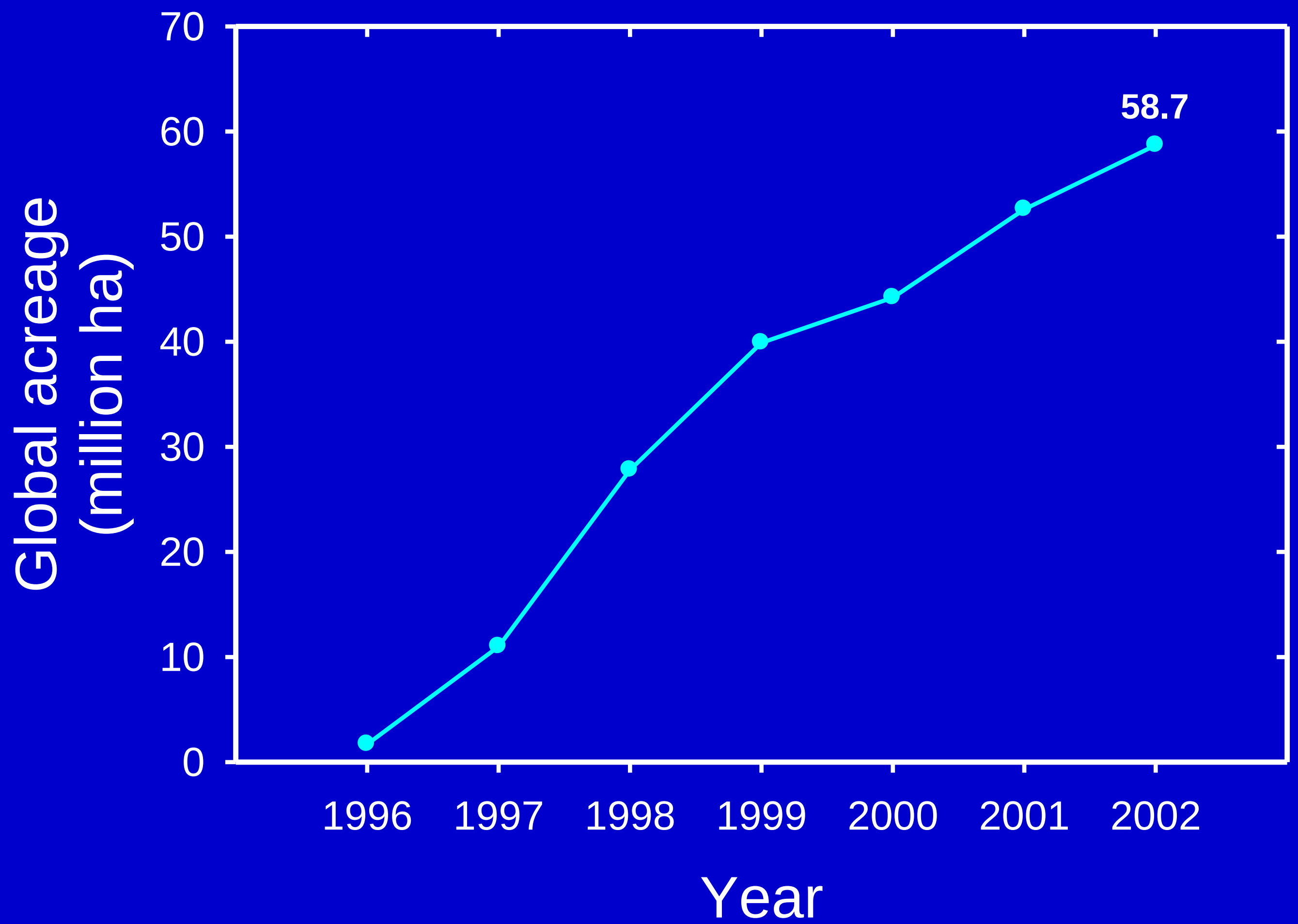
     e.g., Sulfonylurea-tolerant soybean (STS)
     IMI corn

Transgenic and non-transgenic herbicide-resistant corn and soybean

Crop	Soybean	Corn
Transgenic	Roundup Ready, 1996	Roundup Ready, 1998
		RR/YieldGard, 2000
		Liberty Link, 1997
Non-transgenic	STS Soybean (Sulfonylureas), 1994	SR corn (Sethoxydim), 1996
		IMI corn (Imidazolinones), 1993



Adoption of herbicide-resistant corn, cotton, and soybean in USA.
Source: USDA 2002; Gianesi et al. 2003.



Global adoption of transgenic crops, 1996-2002.
Source: ISAAA 2003, Online.

Countries growing transgenic (insect and herbicide resistant) crops in 2002

Country	% of global area
USA	66
Argentina	23
Canada	6
China	4
South Africa, Australia, Romania, Mexico, Bulgaria, Spain, Germany, Uruguay, Indonesia, Columbia	1

James, 2003; www.isaaa.org

Major transgenic (insect and herbicide resistant) crops grown, globally in 2002

Crop	% of global area
Soybean	62
Corn	21
Cotton	12
Canola	5

James, 2003; www.isaaa.org

Dominant traits grown, globally in 2002

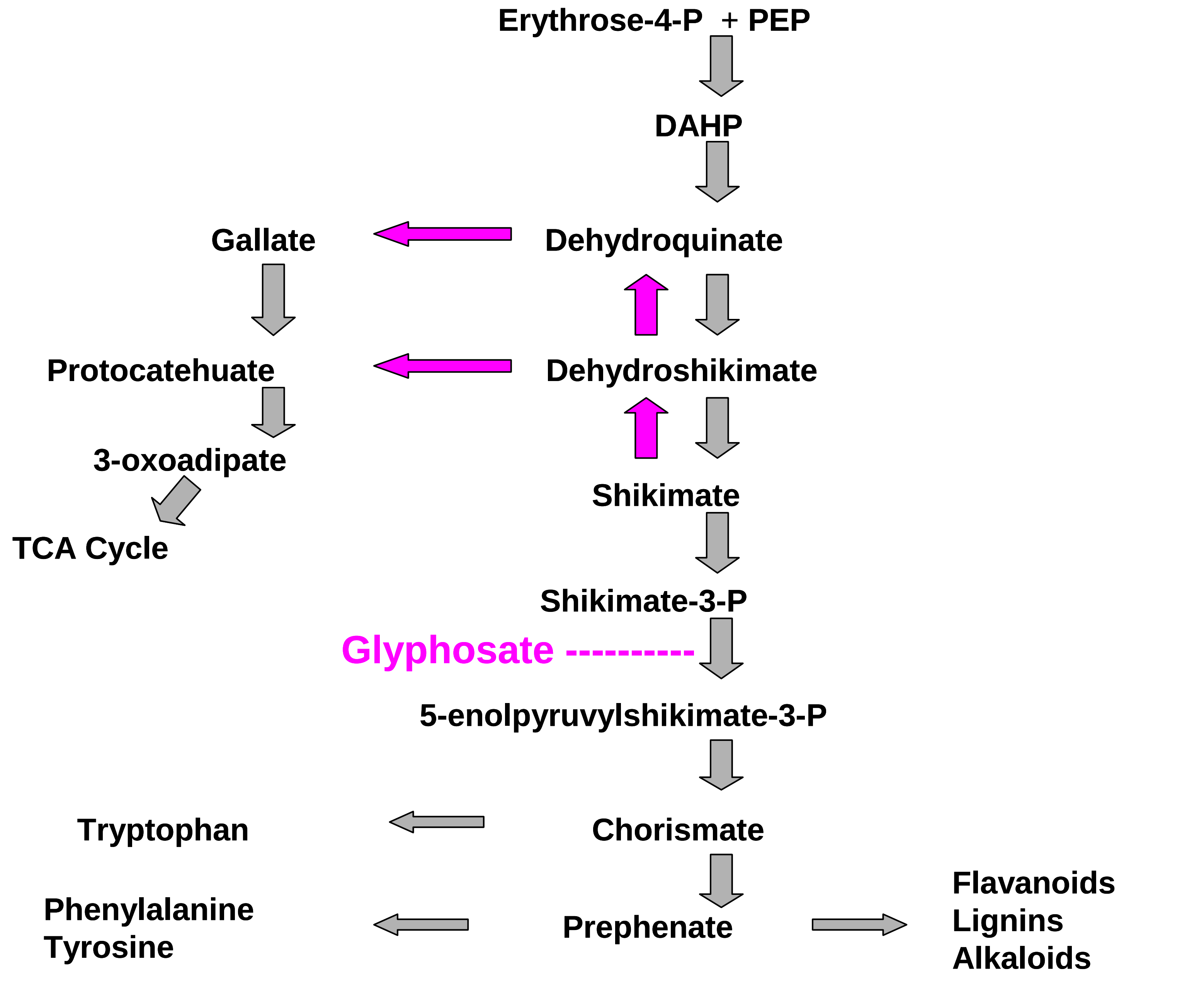
Trait	% of global area	
Herbicide resistant	75	Glyphosate Glufosinate Bromoxynil
Insect resistant, Bt crops	17	
Staked gene, herbicide/Bt	8	

James, 2003; www.isaaa.org

Glyphosate

- Glyphosate [N-(phosphonomethyl)glycine] is a non-selective herbicide
- Extensively used throughout the world for over 20 years
- Foliar-applied herbicide
- Controls a broad-spectrum of annual, biennial, and perennial weeds
- Application rate: 0.21 to 4.2 kg ae/ha

Higher phenolic acids (gallate, protocatechuate) immobalize boron?



Shikimic acid pathway and glyphosate inhibition

Glyphosate

- Glyphosate is the only herbicide reported to inhibit enzyme EPSPS.
- EPSPS is not present in animals (Padgette et al., 1995)
  - Glyphosate is considered an environmentally benign herbicide
- Roundup Ready soybean was developed by inserting *cp4* EPSPS gene from common soil bacterium *Agrobacterium* sp.

Herbicide-Resistant Crops (HRCs) - Benefits

Herbicide-resistant soybean and corn - Benefits

- **Simplicity of weed control**
  - One herbicide controls a wide spectrum of broadleaf, grass, and sedge weeds.
  - Fewer herbicide application trips.
  - In contrast, 3-5 different herbicides are used in Non-GR soybean.
  - Use of one herbicide (glyphosate) eliminates concern for antagonism, which usually associated with tank mixes of grass and broadleaf herbicides.

Glyphosate-resistant soybean and corn - Benefits

Flexibility in glyphosate application rate and timing

Crop	Application rate: Initial	Sequential	Total in-crop	Application timing
	------------------ kg ae/ha	----------------		
Soybean	0.87 - 1.73	0.87 – 1.73	2.52	Emergence to flowering
Corn	0.63 – 0.87	0.63 – 0.87	1.73	Emergence to 8-leaf

Rate: bigger weeds use higher rates
Timing: wider window of application

Glufosinate-resistant corn - Benefits

Flexibility in glufosiante application rate and timing

Crop	Application rate			Application timing
	Initial	Sequential	Total in-crop	
	----------------- kg ae/ha ----------------			
Corn	0.36 – 0.44	0.36 – 0.44	0.81	Emergence to 7-leaf

Rate: bigger weeds use higher rates
Timing: wider window of application

Herbicide-resistant soybean and corn - Benefits

- Two POST applications of glyphosate provide >90% control of most common weeds
  - in GR soybean (Mckinley et al. 1999; Wait et al. 1999; Corrigan and Harvey 2000; Culpepper et al. 2000; Reddy 2001).
  - In GR corn (Tharp and Kells 1999; Johnson et al. 2000).

- Two POST applications of glufosiante provide adequate control of most common weeds in glufosiante-resistant corn (Krausz et al. 1999; Hamill et al. 2000; Ritter and Menbere 2001).

Glyphosate and glufosiante do not control all weed species (partial list)

Weed species	Glyphosate	Glufosiante
Barnyardgrass	G	**F**
Bermudagrass	G	**None**
Crabgrass	E	G
Foxtail	E	E
Johnsongrass	E	**F**
Quackgrass	E	**P**
Canada thistle	G	**P**
Horseweed	**R**	G
Lambsquarters	F-G	**F**
Pitted morningglory	F-G	E
Prickly sida	F-G	E
Sicklepod	F-G	G
Yellow nutsedge	**F**	**P**
Waterhemp	**F**	E

P=poor, F=fair; G=good; E=excellent; R=Resistant.

Glyphosate- and glufosinate-resistant soybean and corn - Benefits

- Can preemergence (PRE) herbicides be eliminated?
  - Yes/No (it depends).
- So far, research has shown 1 or 2 POST ≥ PRE fb 1 POST.
- PRE applications are prophylactic treatments. Well managed farms with low weed pressure – No need for PRE.
- Heavy weed pressure and multiple flushes of weeds – need PRE.
- PRE herbicides widen the window for POST applications of glyphosate and glufosiante.
  - e.g., during extended rainy periods, those farmers who have limited labor and equipment.

Control

Glyphosate POST only

Soybean 2 weeks after 2nd POST application

Standard PRE + Glyphosate POST

C. H. Koger, 6/05/2003

Control

Glyphosate POST only

Standard PRE + Glyphosate POST

Corn 2 weeks after 2nd POST application

K. N. Reddy, 5/19/2003

Glyphosate- and glufosinate-resistant soybean and corn - Benefits

- Do they (glyphosate and glufosinate) need tank mix partners (herbicides)?
  - Depends on weed species.
  - Tank mixing glyphosate and glufosinate with other POST herbicides can enhance control of certain weed species.
- Tank mixing herbicides with residual soil activity can also prevent late-season weed emergence.
  - e.g., chlorimuron, imazaquin with glyphosate in soybean.
  - e.g., atrazine, flumetsulam with glufosiante in corn.

Glyphosate- and glufosinate-resistant soybean and corn - Benefits

- Lower herbicide cost: Glyphosate–based weed control programs cost less than alternatives.

- Reduction in herbicide use (active ingredient)

Economics of weed control in non-irrigated glyphosate-resistant (GR) soybean, 2000- 2002

Soybean type	Herbicide program	Cost of herbicide program*	Soybean yield	Net return
		$/ha	kg/ha	$/ha
GR	PRE + POST	107	2638	186
	POST	75	2628	218
Non-GR	PRE + POST	169	2498	94
	POST	126	2383	119

*Includes technology fee for GR soybean of 21 to 22 $/ha.
Source: Heatherly et al. 2003, Submitted to Agronomy J.

Impact of glyphosate-resistant soybean on herbicide use pattern (partial list) in the USA, 1995 – 2001.

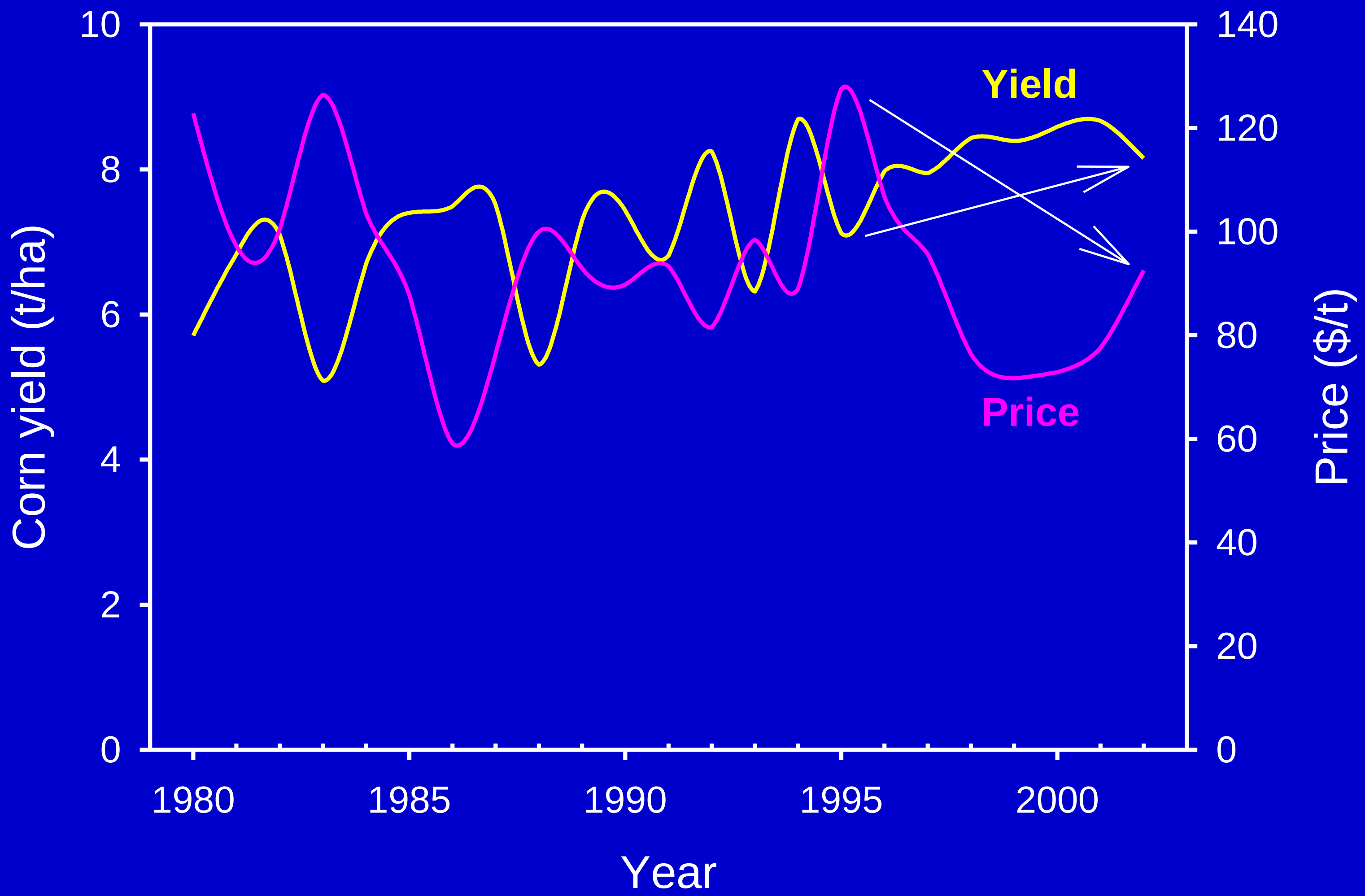
Herbicide	Active ingredient used in soybean		
	1995	1998	2001
	---------------- million kg --------------		
Alachlor	1.78	0.95	0.22 ↓
Bentazon	1.93	1.40	0.19 ↓
Imazethapyr	0.60	0.21	0.10 ↓
Metolachlor	3.17	2.01	0.44 ↓
Metribuzin	0.63	0.38	0.11 ↓
Pendimethalin	5.86	5.32	2.41 ↓
Trifluralin	3.78	4.46	1.46 ↓
Glyphosate	2.86	12.76	14.89 ↑
All herbicides	25.6	32.4	22.9 ↓
Soybean area, mha	25.3	29.1	30.0

Source: USDA, NASS 2003, Online.

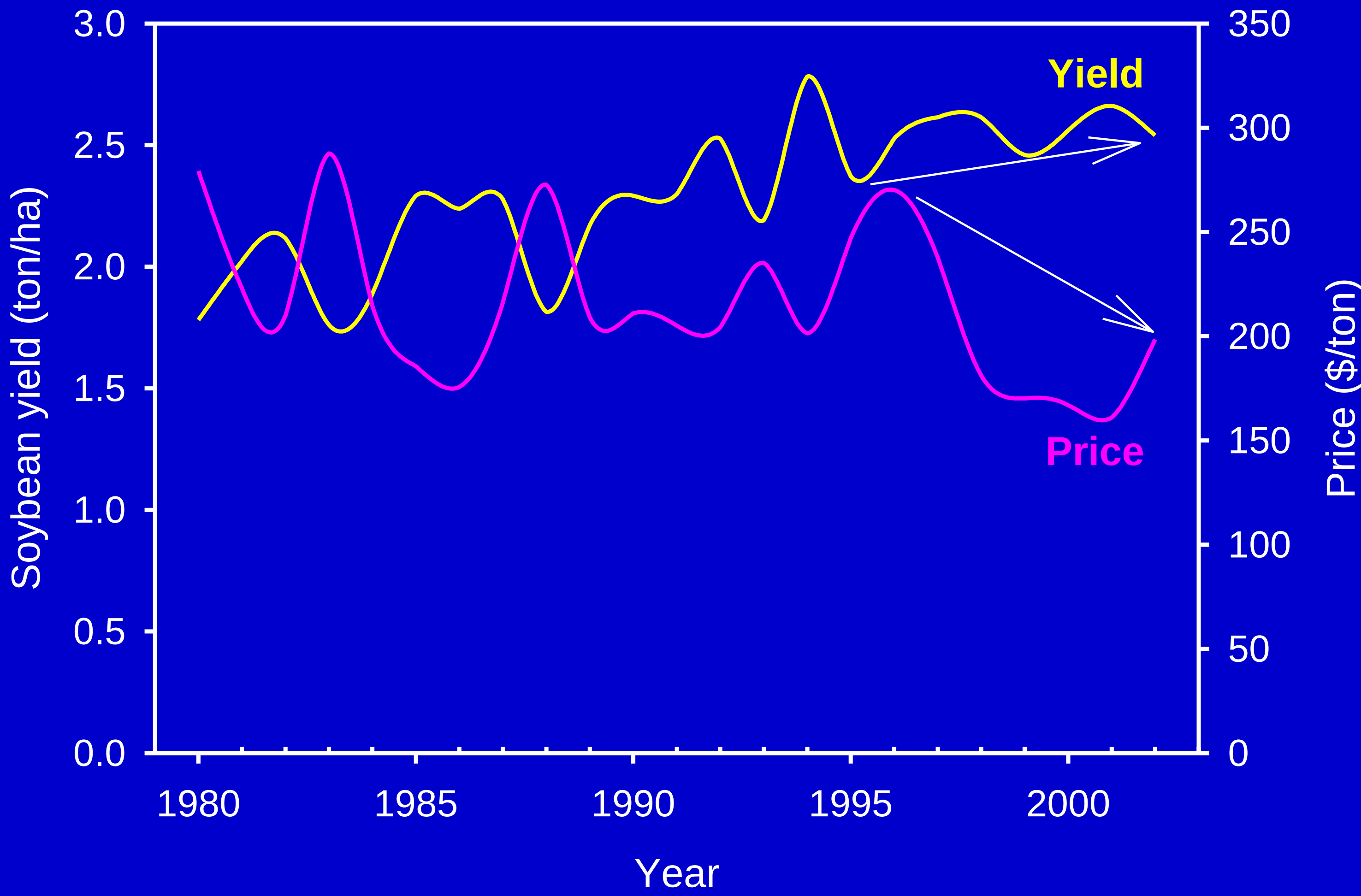
Herbicide-resistant transgenic corn and soybean impact on herbicide use and cost in the USA in 2001

Crop	HRC acreage	Reduction		
		Herbicide use	Herbicide costs	Cost savings/ha
	ha	million kg	million $	$/ha
Corn	2.4	2.6	58.0	25
Soybean	20.2	13.0	1011	50

Source: Gianessi et al. 2002. www.ncfap.org/40CaseStudies.htm



Trends in corn yields and prices in the USA, 1980 - 2002.
Source: USDA Online.



Trends in soybean yields and prices in the USA, 1980 - 2002.
Source: USDA Online.

Herbicide-resistant soybean and corn - Benefits

- There are 166 weeds species (99 dicots and 67 monocots) have evolved resistance to one or more herbicides in 59 countries (Heap 2003; www.weedscience.com).
- Glyphosate- and glufosinate-resistant crops offer an option in the management of weeds resistant to other herbicides.
  - e.g., common cocklebur resistant to ALS-inhibitors and organoarsenicals.
  - Johnsongrass resistant to dinitroanilines and ACCase-inhibitors.
  - Pigweeds resistant to ALS–inhibitors.

Herbicide-resistant soybean and corn - Benefits

- No crop rotation restrictions.
  - In Non-GR crops, many herbicides have planting restrictions intervals for a rotational crop.
    - For example:
    - Halosulfuron used in corn has 10 m for soybean.
    - Imazaquin in soybean has 9.5 m for corn.
    - Chlorimuron in soybean has 9 m for corn.
    - Clomazone used in soybean has 9 m for corn.
  - Freedom to rotate GR soybean and corn

Herbicide-resistant soybean and corn - Benefits

- **HRCs have encouraged adoption of conservation tillage.**
- Conservation tillage systems (>30% of soil surface covered with crop residue, after planting) offer numerous benefits.
- Adoption of conservation tillage began to level off somewhat by mid-1990s.
- However, since mid-1990s, farmers have been moving toward conservation tillage system.
  - Primary reason for tillage is weed control.
  - With HRCs, farmers allow weeds to emerge with their crops and then control weeds with POST applications of highly effective broad-spectrum herbicides without harming the crop.
  - Perennial weeds often associated with conservation tillage systems can now be controlled with glyphosate in GR crops.
  - Improved weed control capability with HRCs gave increased confidence in farmers to control weeds economically without relying on tillage.

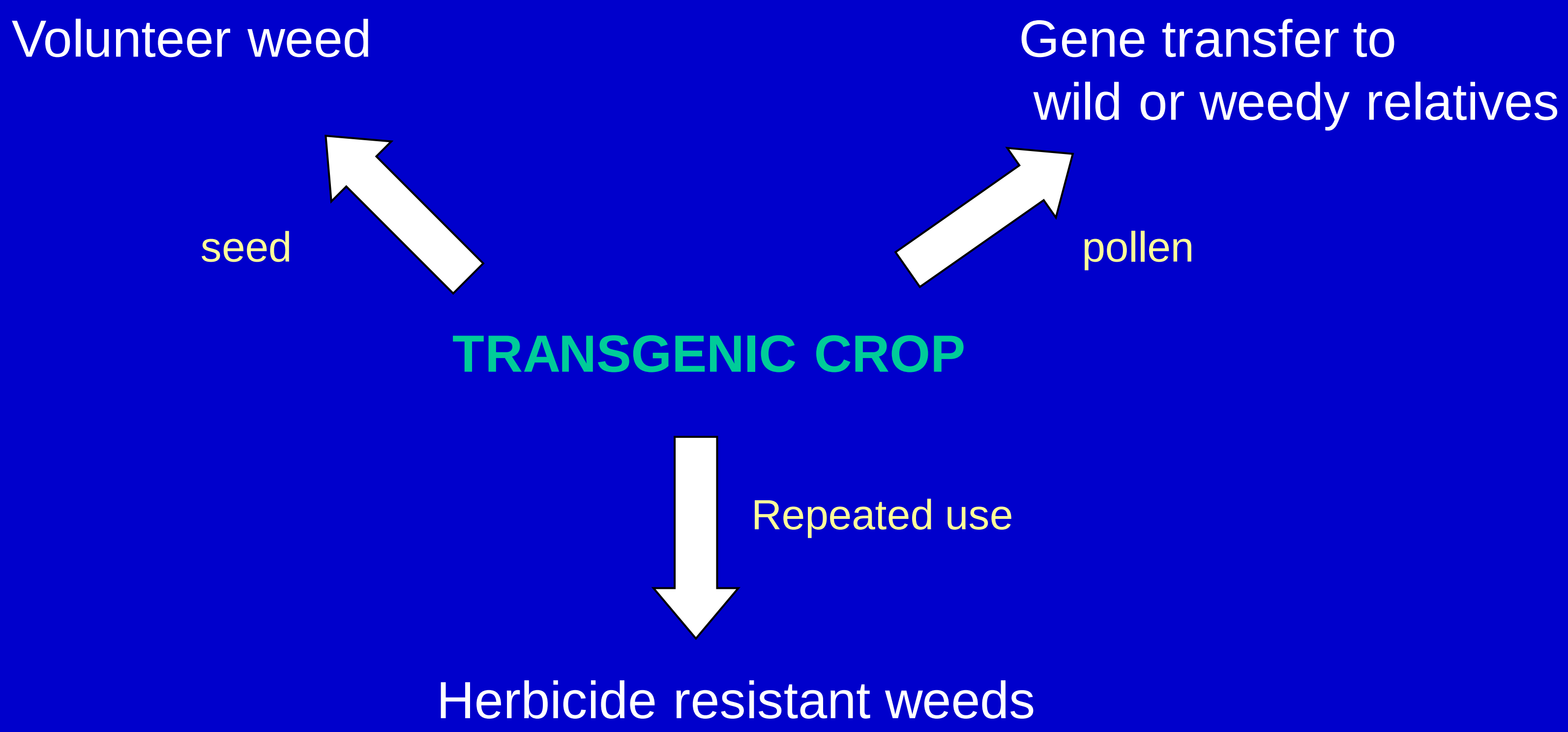
Adoption of glyphosate-resistant soybean and corn by tillage system in the USA, 1998-2000

Crop/Year		Conventional tillage	Reduced tillage	No-till
		Percent of area planted to glyphosate-resistant crop		
Soybean				
	1998	29	35	51
	1999	47	56	71
	2000	53	64	75
Corn				
	1998	1	1	2
	1999	3	3	4
	2000	4	4	7

Source: Fawcett and Towery 2003; www.ctic.purdue.edu

Herbicide-Resistant Crops (HRCs) - Risks

Herbicide-resistant crops - Potential risks



Herbicide-resistant soybean and corn - Potential risks

- Volunteer plants of a previous crop – become weed in succeeding crop.
  - In a corn-soybean and corn-cotton rotation, farmers planting GR soybean or GR cotton after GR corn will not be able to control volunteer GR corn with glyphosate.
  - Volunteer plants of one GR soybean variety can be a weed problem in seed production of another GR soybean variety.
- Control of volunteer plants requires alternative strategy or other herbicides.

Herbicide-resistant soybean and corn - Potential risks

- Weed Species and population shifts
- Certain weed species are naturally tolerant to glyphosate/glufosiante.
- Repeated applications of glyphosate/glufosinate can exert selection pressure and cause build up of best adapted weed species and biotypes.
  - Weed species shift: from less to more tolerant species.
  - Population shifts: increase in frequency of a given species.
  - Reports of lack of control of common waterhemp (*Amaranthus rudis*) by glyphosate in soybean (Owen 1998).
  - 3 yr of continuous BXN cotton resulted in weed shift towards common purslane, sicklepod, and yellow nutsedge (Reddy 2003).

Herbicide-resistant soybean and corn - Potential risks

- Evolution of weed resistance:
- Continuous use of a single herbicide (or herbicide with same mode of action) leads to selection of resistant weed populations.

  – Monoculture using herbicide with the same mode of action.
    - e.g., continuous GR soybean.

  – Crop rotation using herbicide with the same mode of action.
    - e.g., glyphosate-resistant soybean rotated with glyphosate-resistant corn.

  – The above practices will more likely increase the selection pressure on certain weed populations especially when no PRE herbicides are used.

Herbicide-resistant soybean and corn - Potential risks

- **Glyphosate-resistant weeds:**

- Naturally occurring resistant weeds.
  - Field bindweed (*Convolvulus arvensis*) – DeGennaro and Weller 1984.
  - Birdsfoot trefoil (*Lotus corniculatus*) – Boerboom et al. 1990.

- Evolved resistant weeds (due to repeated use of glyphosate).
  - 1996 - Rigid ryegrass (*Lolium rigidum*) – in Australia, USA, South Africa.
  - 2001 - Italian ryegrass (*Lolium multiflorum*) in Chile.
  - 1997 - Goosegrass (*Eleusine indica*) in Malaysia.
  - 2000 - Horseweed (*Conyza canadensis*) in USA.

Herbicide-resistant soybean and corn - Potential risks

- **Glufosinate-resistant weeds:**

- No known resistant weeds, yet.

Herbicide-resistant crops - Potential risks

- Gene movement between crop plants and related wild and weed species is possible (Raybould and Gray 1994; Dale 1994; Warwick 1997).
- For example:
  - *Beta vulgaris* (sugar beet) to wild beet
  - *Brassica napus* (canola, rapeseed) to *B. rapa* (wild rape)
  - *Sorghum bicolor* (Sorghum) to *S. halepense* (johnsongrass)
  - *Zea mays* (maize, corn) to *Z. mexicana* (Schrad) Kuntze (teosinte)

Herbicide-resistant crops - Potential risks

- **Soybean**
  - *Glycine max* - cultivated
  - *Glycine soja* - wild Grow naturally in Asia and Australia
  - *Glycine gracilis* - weedy Grow naturally in Asia and Australia
  - These cultivated, wild, and weedy forms are “generally interfertile”
- Self pollination
- Cross pollination is $< 1\%$
- No wild and weedy relatives in the USA (except at University and specialized research stations), therefore, no potential for gene movement.

Herbicide-resistant crops - Potential risks

- **Corn**
  - *Zea mays* – cultivated corn and teosinte
  - *Zea diploperennis* – diploperennial teosinte
  - *Zea luxurians* – perennial teosinte
  - *Zea perennis* – perennial teosinte
  - *Zea mexicana* – teosinte, wild grass in Mexico and Guatemala
- Self pollination and cross pollination are possible
  - Frequencies of self vs.cross pollination depends on physical proximity, pollen viability, wind, etc.
- Potential for gene movement through pollen to wild relatives exist.

Herbicide-resistant soybean and corn - Potential risks

- Herbicide drift.
- Herbicide drift injury to off-target crops.
  - This is true with all herbicides.
  - Restrictions on aerial applications?

Herbicide-resistant soybean and corn - Potential risks

- HRCs have negative impact on the development of new herbicides.
  - Glyphosate-resistant crops have markedly impacted herbicide use patterns.
  - Glyphosate use has increased rapidly with a concomitant decrease in the use of other herbicides.
  - In response to lower cost of glyphosate-based weed control programs, other agrochemical industries have dropped the price of their herbicides to remain competitive.
  - Both herbicide market and profit margin are shrinking.
  - Discovery and development of new products is expensive (and time consuming).
  - Currently, fewer herbicides in the ‘pipeline’.
  - Acquisitions of seed companies and mergers of agrochemical industries will reduce the competition for discovery and development of new herbicides.

HRCs - Perspective

- HRCs represent a revolutionary breakthrough in weed control technology.
- HRCs should not be relied on solely to the exclusion of other weed control methods, and should be used within integrated weed management systems.
- Thus, prudent use of HRCs in combination with other weed control methods will most likely prolong their use as a weed management tool.

Glyphosate effects on glyphosate-resistant (GR) soybean physiology

Glyphosate-resistant (GR) soybean injury

- Application of glyphosate to GR soybean has caused injury under certain conditions and with certain salt formulations of glyphosate.
- Injury symptoms:
  - Yellowing
  - Speckling
  - Necrosis
- Injury ranged from 8 to 38%.

Reddy et al. 2000; Reddy and Zablotowicz 2003

Tms (Touchdown 5)

Adt (Engame)

Dia (Touchdown IQ)

Ipa (Roundup Ultra)

Soybean injury, 2 d after 2nd POST

Tms (Touchdown 5)

Adt (Engame)

Dia (Touchdown IQ)

Ipa (Roundup Ultra)

Soybean 14 d after 2nd POST

No injury on newly developed leaves

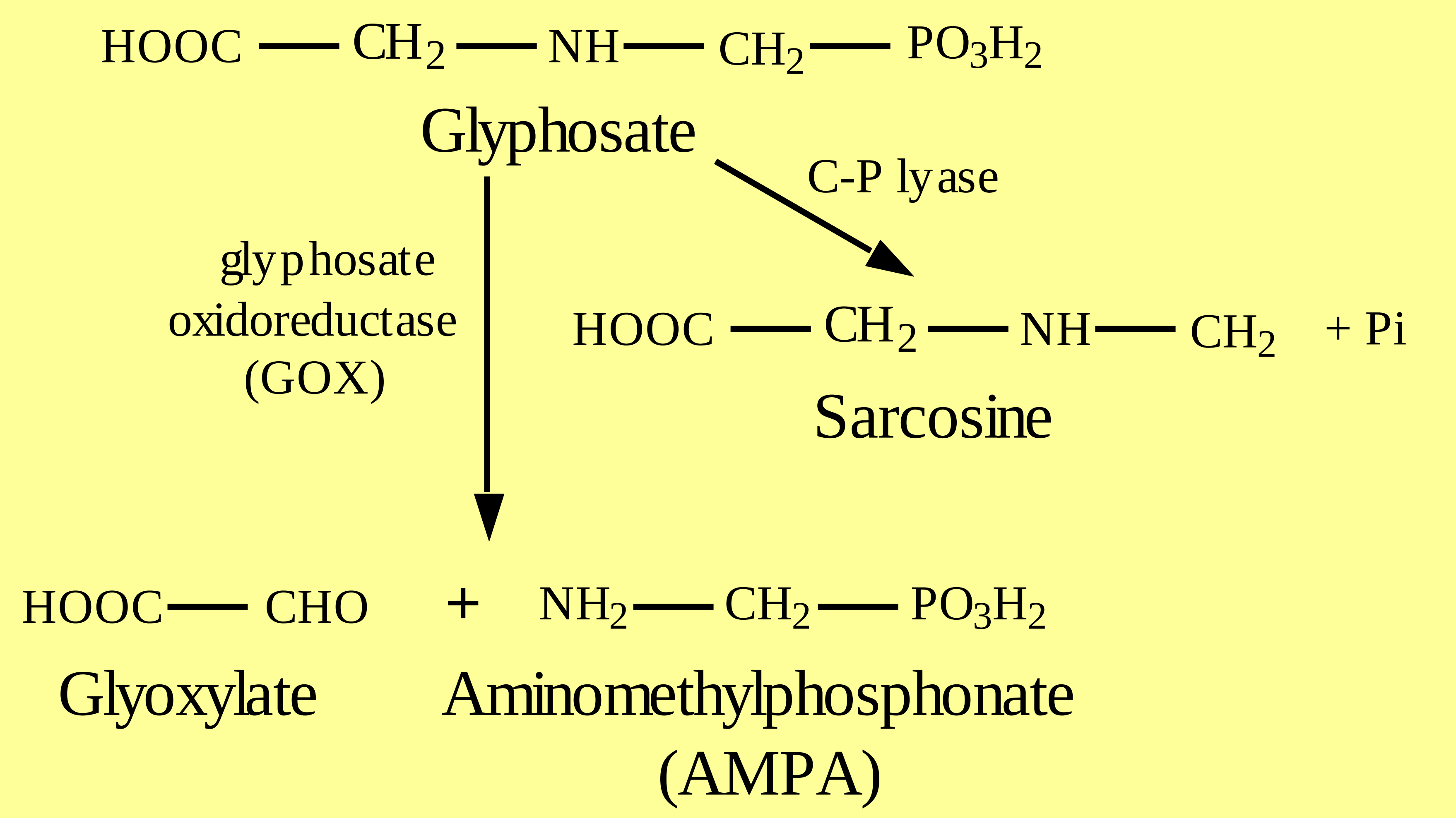
Control

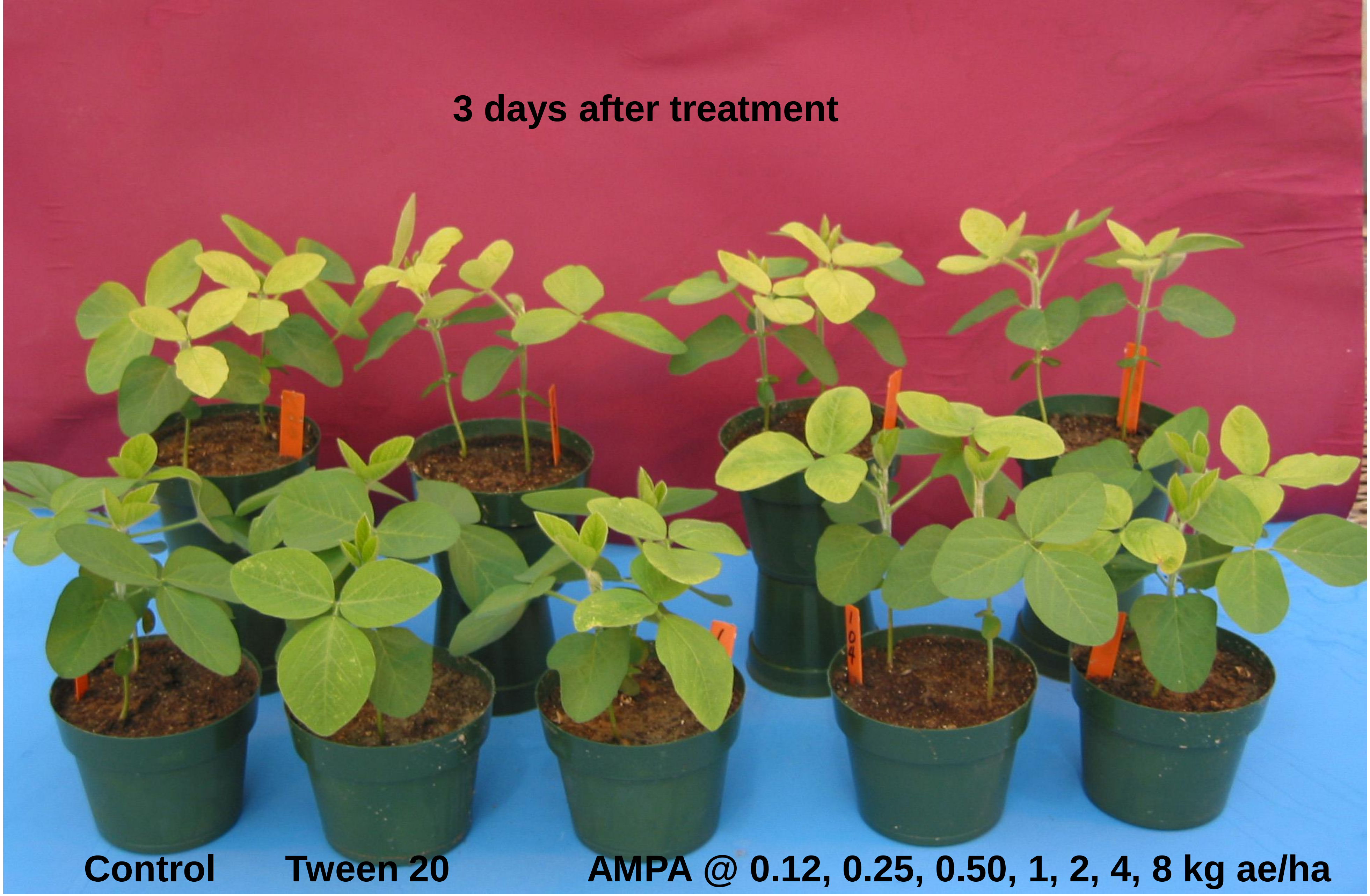
Tween 20

Glyphosate
6.7 kg ae/ha
+ Tween 20

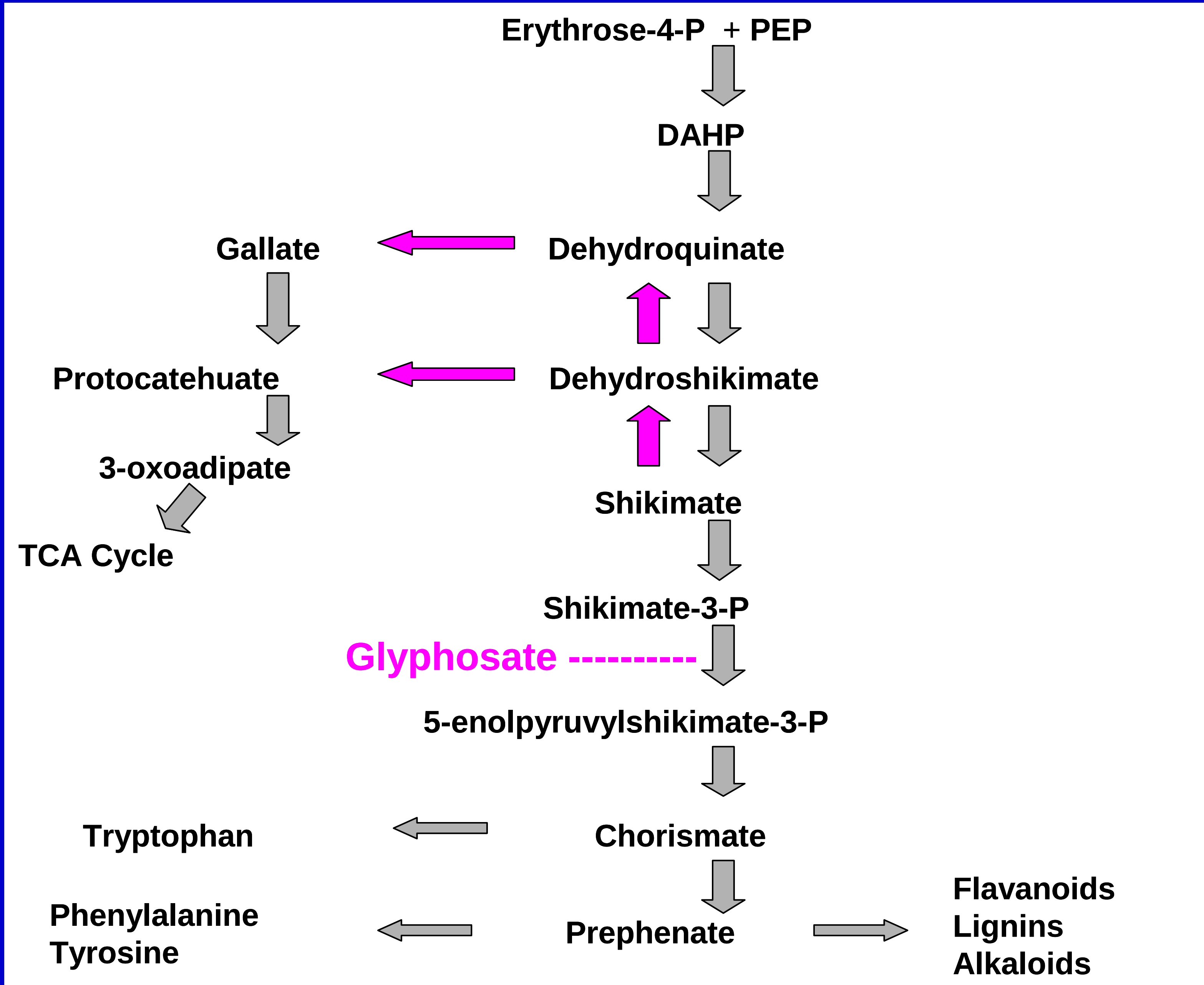
Reddy, Rimando, and Duke, Unpublished

Glyphosate Metabolism





Reddy, Rimando, and Duke, Unpublished



Shikimic acid pathway and glyphosate inhibition

Isoflavone, glyphosate, AMPA levels in seeds of glyphosate-treated, glyphosate-resistant (GR) soybean

- Field studies conducted in 2000 at 2 locations
  - Stoneville, Mississippi and Columbia, Missouri.
  - 4 commonly used glyphosate treatments were compared to hand-weeded control.
  - After harvest, soybean seeds were analyzed.
- **Glyphosate** concentration ranged from 0.1 to 3.1 µg/g seed.
  - USEPA tolerance level is 5 µg/g seed.
- **AMPA** concentration ranged from 0.1 to 25 µg/g seed.
  - No USEPA tolerance levels for AMPA in soybean.
- **Shikimate** levels slightly increased.
  - This indicated that *CP*4 EPSPS utilized all or most of the shikimate that would have accumulated from inhibition of the native EPSPS.
  - If so, one would expect no effect of glyphosate on isoflavones.
- **Isoflavones** (daidzein, daidzin, genistein, genistin, glycitein,glycitin)
  - Glyphosate had no effect on these nutraceutical compounds.

Duke et al. 2003

Glyphosate effects on soil bacteria

- Several species of bacteria metabolize glyphosate as their sole source of phosphate. For example,
  - *Pseudomonas* sp. (Jacob et al. 1988)
  - *Athrobacter* sp. (Pipke et al. 1987)
  - Certain members of Rhizobiaceae - *Rhizobium trifolii*; *Agrobacterium rhizogenes, etc. (Liu et al. 1991)*

- Despite the ability of certain *R. trifolii* strains to detoxify glyphosate, application of glyphosate to the root zone inhibited nodulation and acetylene reduction activity in subterranean clover (Eberbach and Douglas 1989).

Glyphosate effects on *Bradyrhizobium japonicum*

- The soybean nitrogen fixing symbiont, *Bradyrhizobium japonicum* possesses a glyphosate-sensitive EPSPS enzyme.
  - Upon exposure to glyphosate accumulate shikimic acid and protocatechuic acid.
  - Inhibition of growth at low concentrations (0.5 mM glyphosate).
  - Death at high concentrations (5 mM glyphosate).

Jaworski 1972; Moorman et al. 1992; Hernandez et al. 1999

Glyphosate effects on glyphosate-resistant (GR) soybean nodulation

- Glyphosate inhibited nodulation and nodule leghemoglobin content in GR soybean (Reddy et al. 2000; Reddy and Zablotowicz 2003).

- Glyphosate accumulated in nodules of field grown GR soybean (Reddy and Zablotowicz 2003).

- In greenhouse study (King et al. 2001):
  - Nitrogenase activity of GR soybean following glyphosate application was transiently inhibited in early growth stages
  - Higher inhibition under moisture stress.

- In field study - in progress (Zablotowicz and Reddy):
  - 2002: Nitrogenase activity reduced 1 out 6 sampling times. Moisture stress.
  - 2003: No reduction in nitrogenase activity. Good moisture.

Glyphosate effects on nitrogen fixation in glyphosate-resistant (GR) soybean

- Most soybean farmers in the lower Mississippi delta do not use supplemental rhizobium culture or nitrogen fertilizer in soybean production.
  - Soils have higher organic matter
  - Subtle reduction in N_2 fixation may not affect soybean yield

- Effect of glyphosate on N_2 fixation potential of GR soybean in sandy soils and in tropical climate with limited nitrogen availability merits investigation.

- Genetically modified *B. japonicum?*
  - Resistant EPSPS gene
  - C-P lyase gene to metabolize glyphosate.

cultivar resistant to charcoal rot　　　　cultivar susceptible to charcoal rot

Courtesy: A. Mengistu, USDA

Charcoal rot (*Macrophomina phaseolina*) populations in soil as affected by tillage and cover crops in glyphosate-resistant soybean at harvest, 2002

Tillage	Cover crop	*M. phaseolina* populations in soil at harvest: Glyphosate	Non-glyphosate
		Colony forming units/g soil	
Conventional till			
	None	14	13
	Hairy vetch	11	9
	Rye	11	15
No-till			
	None	13	14
	Hairy vetch	23	17
	Rye	27	19

Mengistu and Reddy, unpublished

Glyphosate effects on fluorescein diacetate hydrolytic activity (FDA) in soil as affected by tillage in glyphosate-resistant soybean following two applications, 2002

	1 week after 2nd POST	
Tillage	**Glyphosate**	**Non-glyphosate**
	nmol fluorescein/g soil/h	
Conventional tillage	114	117
No-tillage	211	196

Note: FDA is a measure of soil enzyme esterase and is an indicator of both microbial activity and microbial biomass.

Zablotowicz and Reddy, unpublished

Glyphosate effects on soil bacterial and fungal populations as affected by tillage in glyphosate-resistant soybean following two applications, 2002

Tillage	Total bacteria		Total fungi	
	Glyphosate	Non-glyphosate	Glyphosate	Non-glyphosate
	------------- log_{10} colony forming units/g soil -----------			
Conventional tillage	7.88	7.92	5.20	5.41
No-tillage	7.95	7.92	5.62	5.64

2002 - 1st year of study.
2003 samples are being processed.

Zablotowicz and Reddy, unpublished

Glyphosate effects on glyphosate-resistant (GR) soybean yield

- Extensive research under a wide range of environments indicated no yield reductions due to glyphosate applications on GR soybean compared to soybean treated with standard non-glyphosate herbicides.
  - e.g., Delannay et al. 1995; Elmore et al. 2001; Krausz and Young 2001; Nelson and Renner 1999, 2001; Reddy and Whiting 2000.

- One study by Elmore et al. (2001) showed that GR sister lines yielded 5% less than non-GR sisters.

- Currently, hundreds of GR soybean cultivars are commercially available with yield potential equal or greater than non-GR cultivars.


USDA-ARS
ONE WAY