Acharya N. G. Ranga Agricultural University

class seminar on

HERBICIDE RESISTANCE

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BAD-14-06

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RESISTANCE

- The opposition offered by one thing, force, etc., to another.

WEED:

- A **weed** is a plant considered undesirable in a particular situation

What type of compounds are herbicides?

- Compounds that affect plant specific biological processes
- Many of the targeted pathways are located in the chloroplast
- Herbicidal activity of many herbicides result from the specific inhibition of a single enzyme/ protein

First Resistant Weeds

- ✓ Resistant weeds are nothing new
  - ✓ Wild Carrot – 2,4-D; Canada 1963
  - ✓ Common Groundsel – Atrazine; Washington St. 1970

DEFINITION

Inherited ability of a weed or crop biotype to survive a herbicide application to which the original population was susceptible.

Biotype = a group of plants within a species that has biological traits that are not common to the population as a whole.

Herbicide Resistance

- **Cross resistance**

  Weed biotype that has gained resistance to more than 1 herbicide with the same mode of action but same or different families.

- **Multiple resistance**

  Weed biotype that has developed tolerance to more than one herbicide brought about by different selection pressures *(different modes of action)*.

Development of herbicide resistance

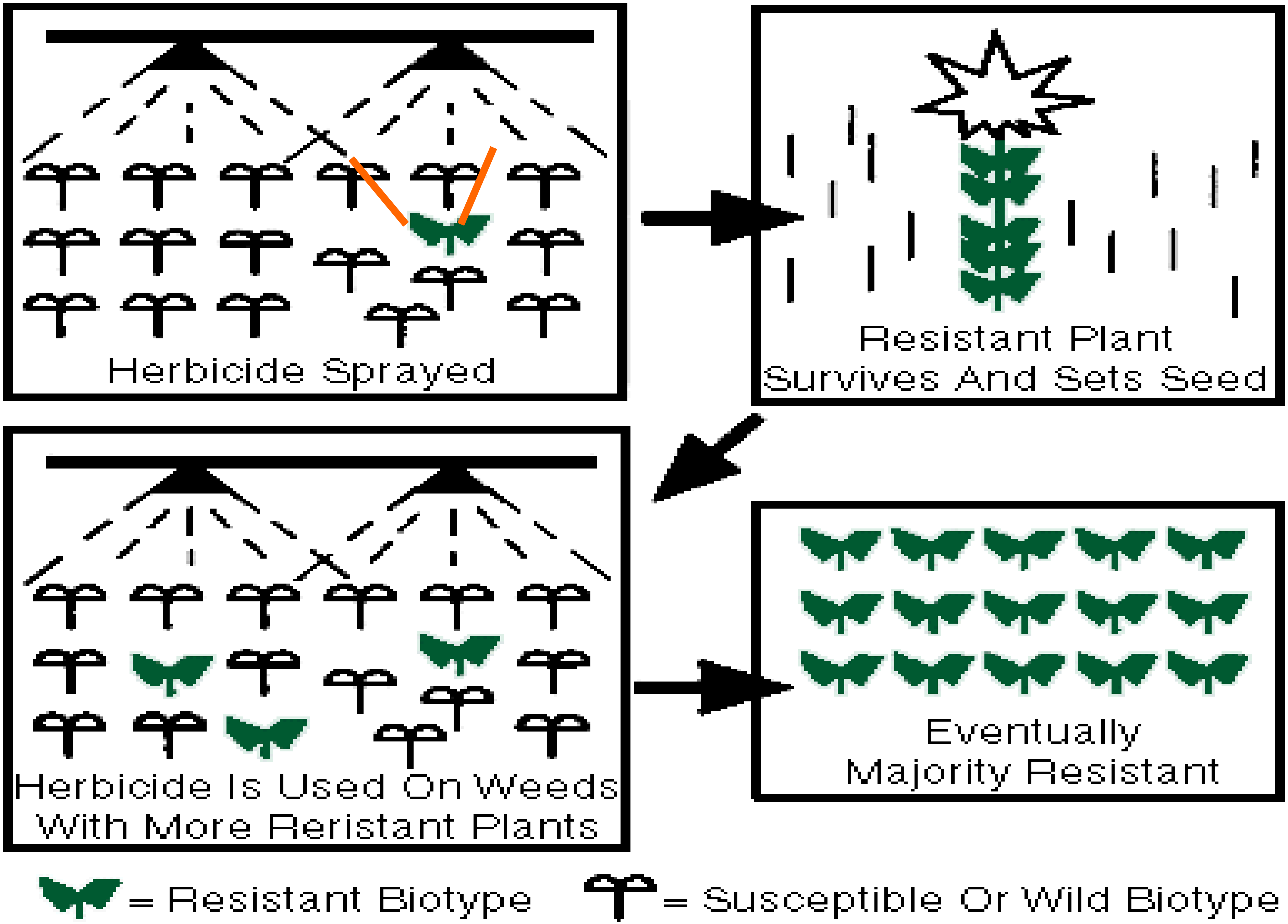
Following favours herbicide resistance

- **Repeated use of a specific herbicide** or a combination of herbicides
- Weed populations with **wide genetic diversity** may develop resistance rapidly, especially for herbicides with a single mechanism of action
- Weed possessing characters like large plant numbers, **prolific seed production**, high rates of weed **migration**/spread, and **diverse environmental conditions** may contribute to **high genetic diversity** **and** develop resistance very quickly

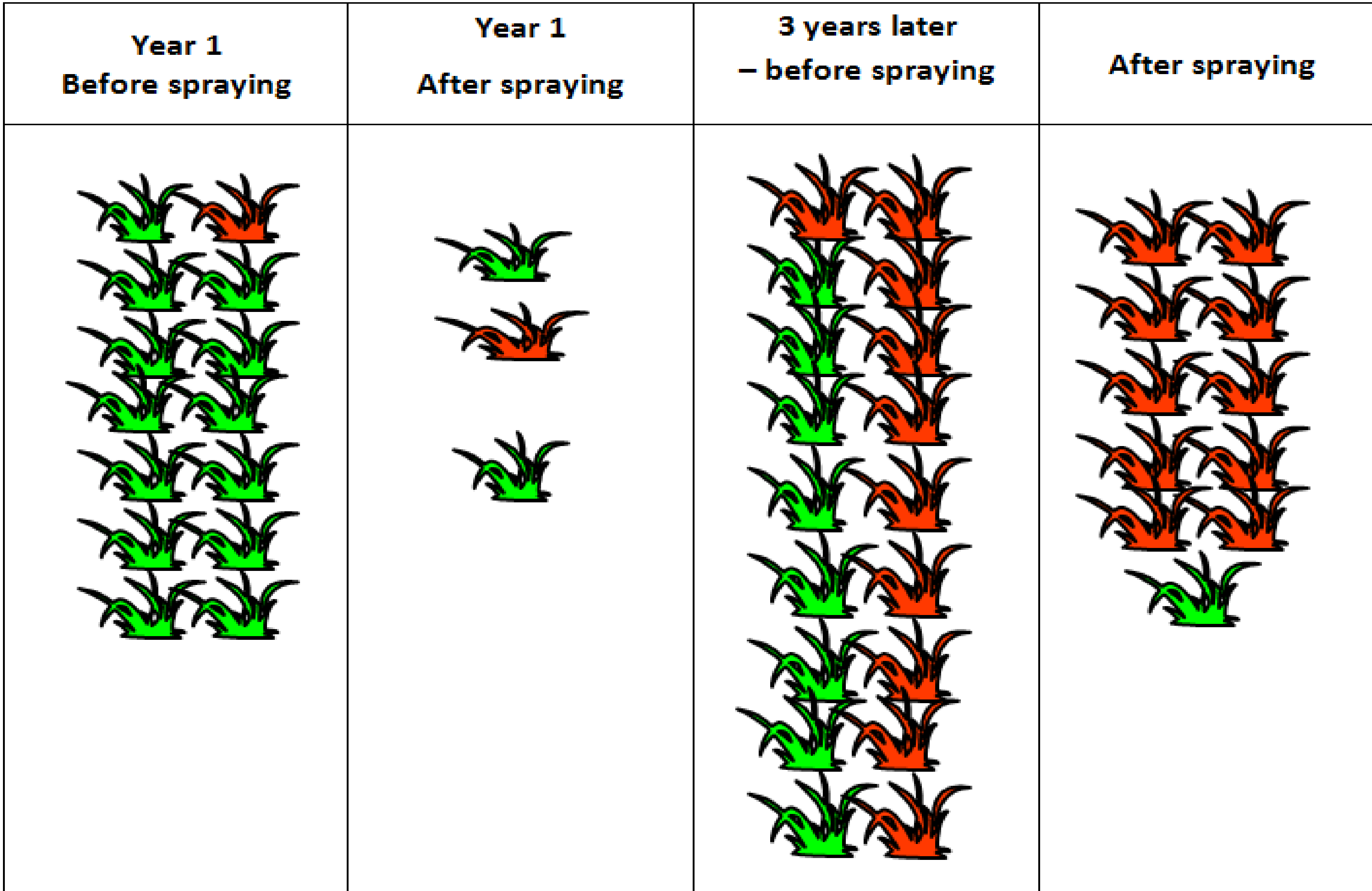
Where do Resistant Weeds Come From?

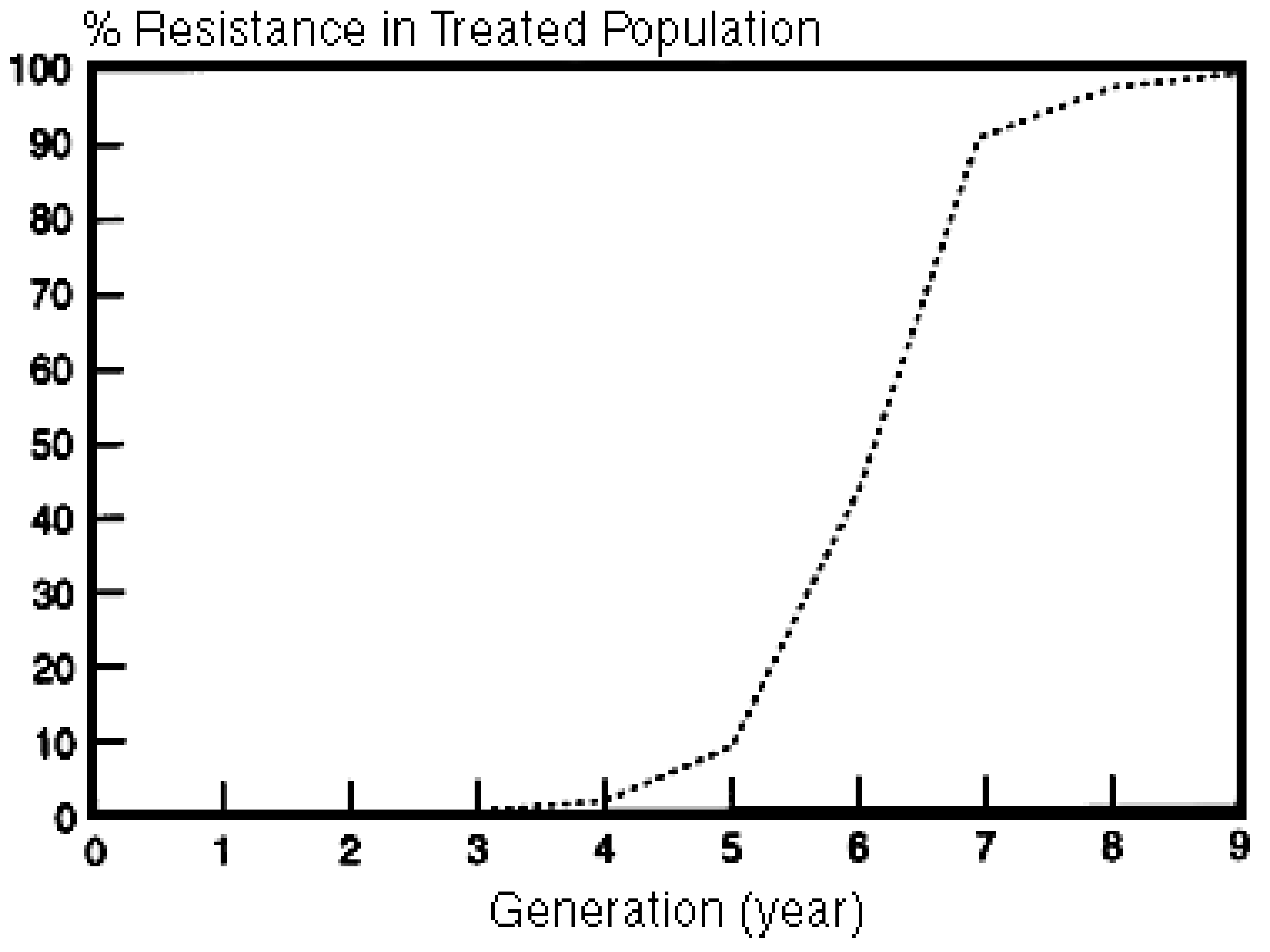
It's all about selection.....

How Does Selection For Herbicide Resistance Occur?

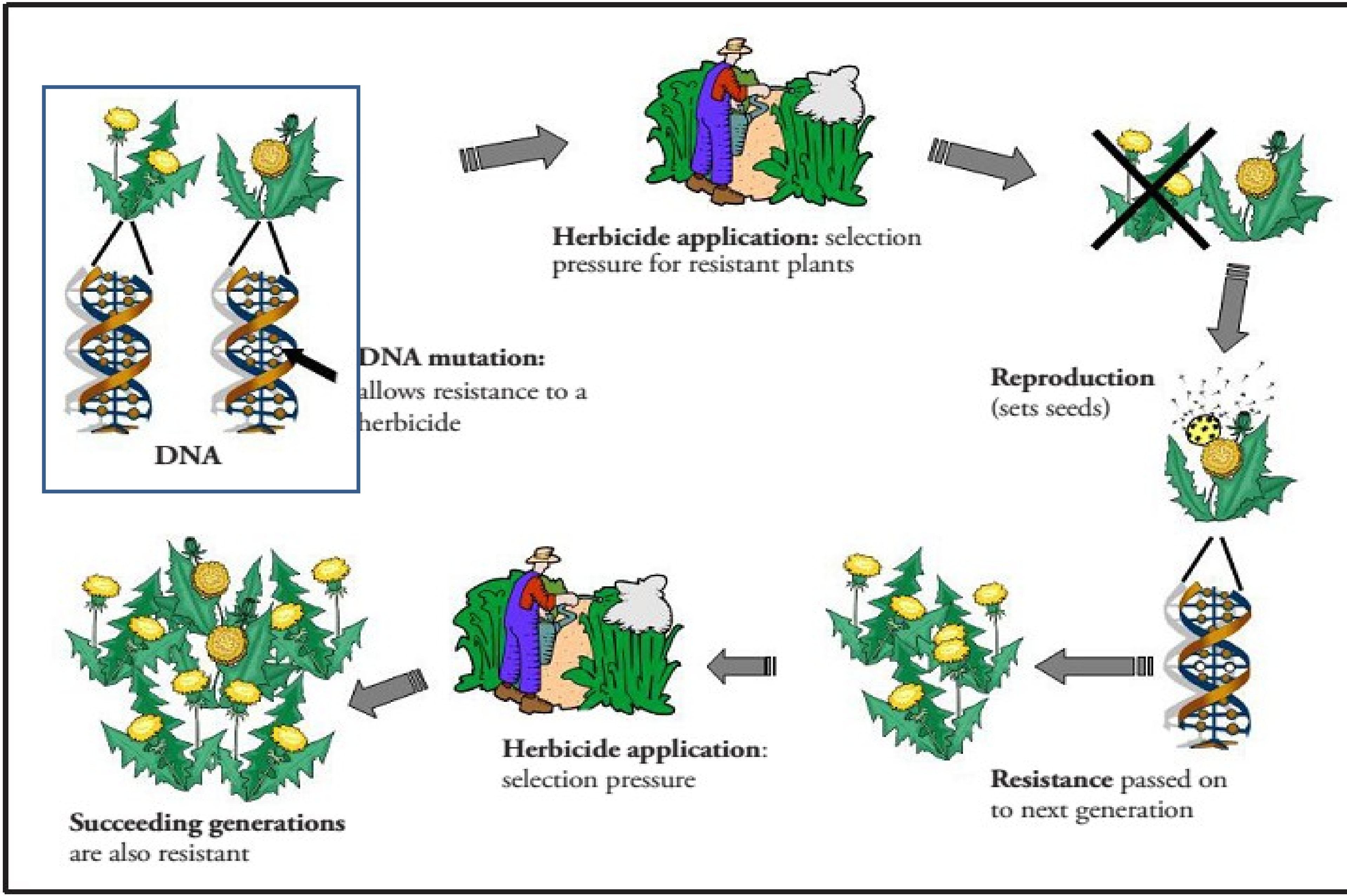


Development of herbicide resistance




% Resistance in Treated Population
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
Generation (year)

Where do resistant weeds come from?



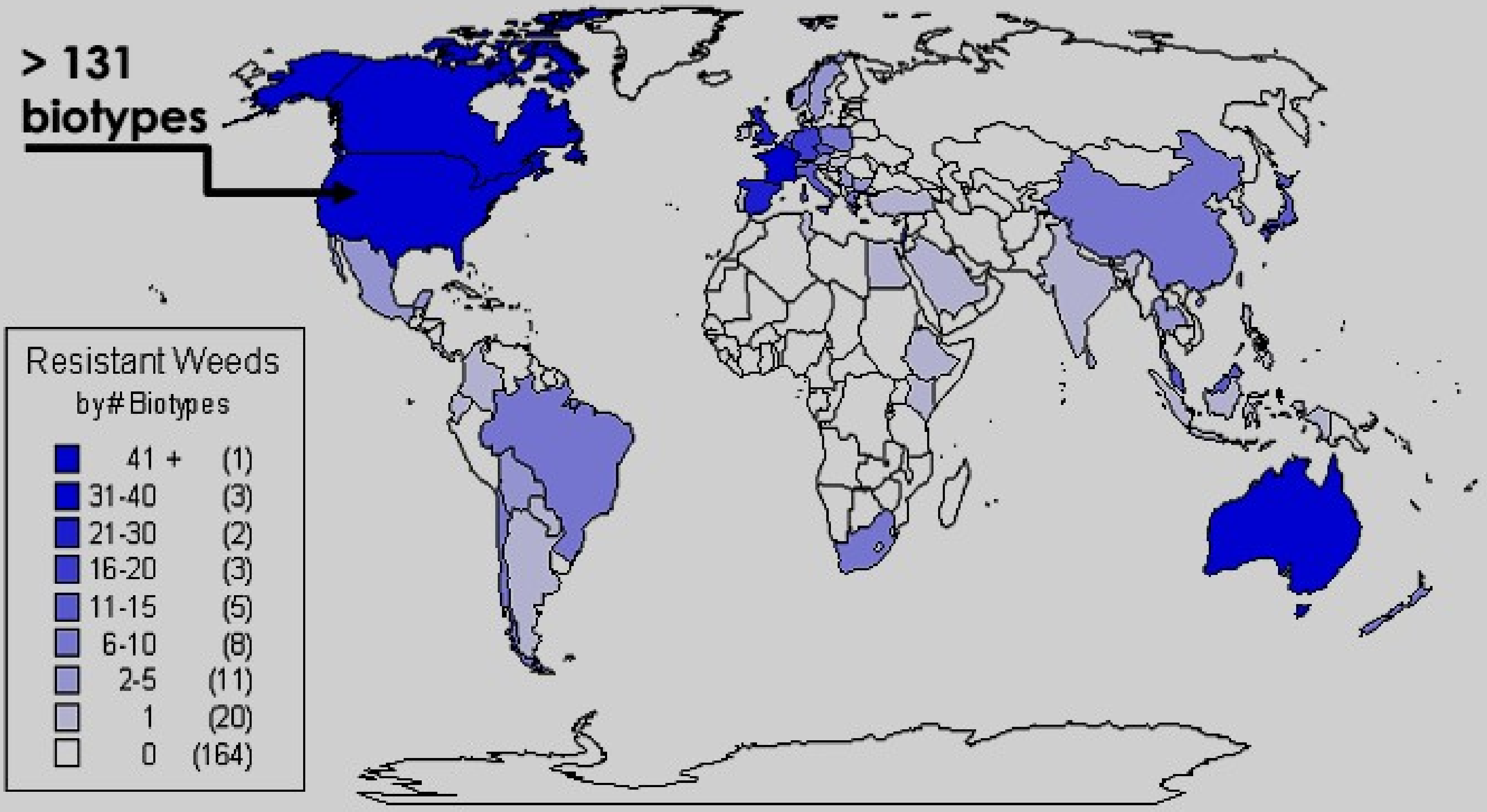
Is it a serious issue?

December 21, 2012:

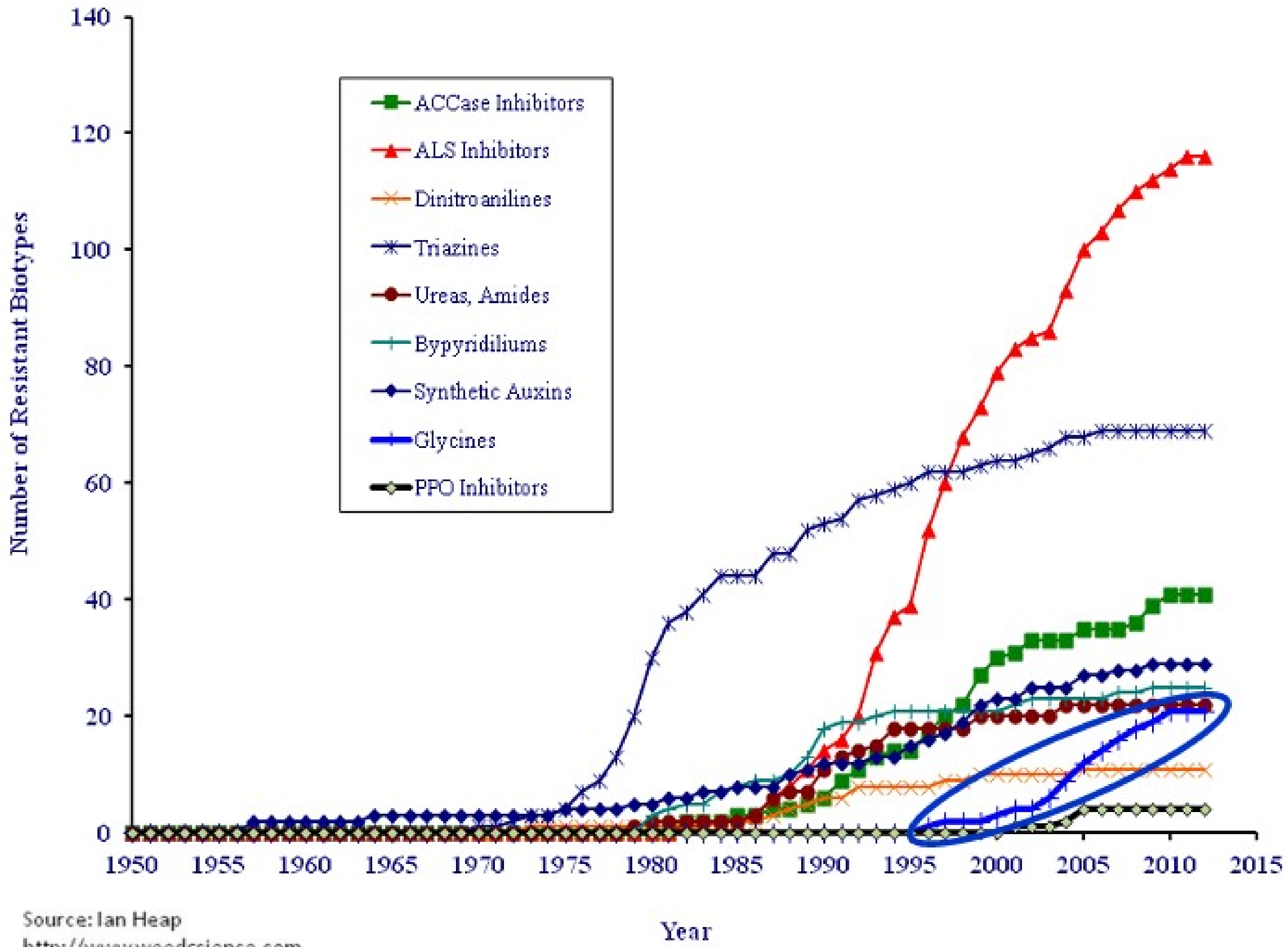
- 396 Resistant Biotypes,
- 210 Species (123 dicots and 87 monocots)

Source: International survey of herbicide resistant weeds (www.weedscience.org)

Distribution of Herbicide Resistant Biotypes

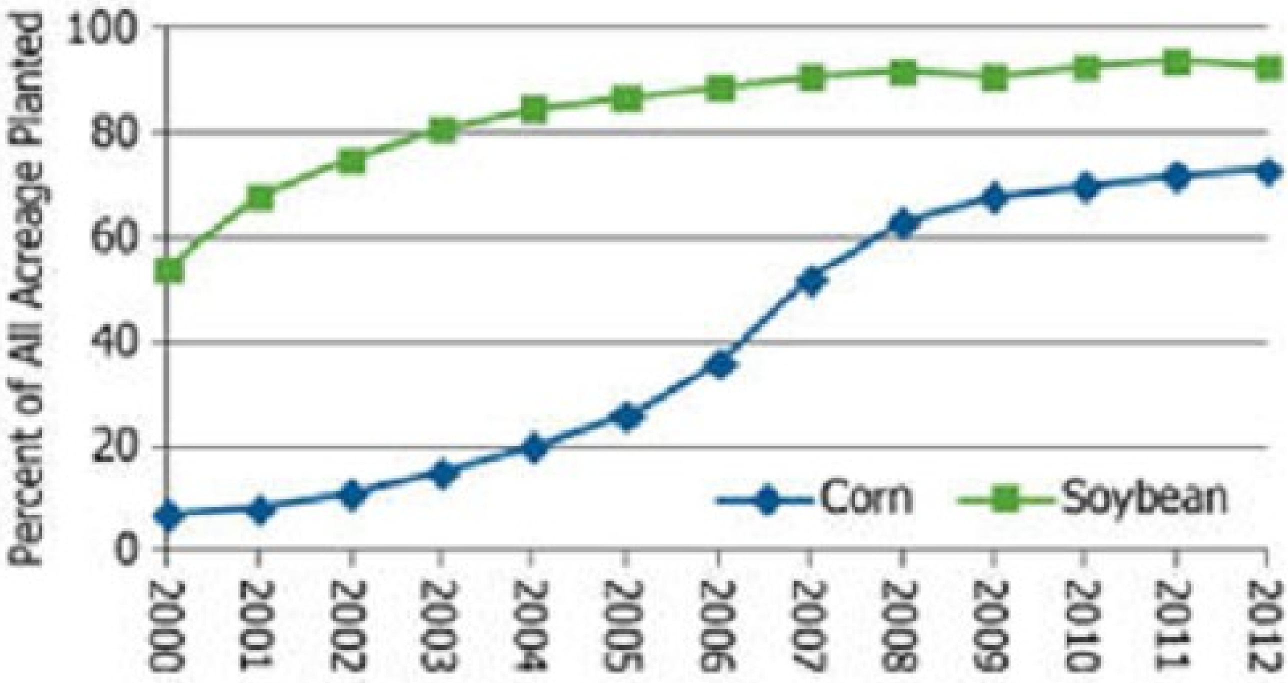


Source: Dr. Ian Heap
www.weedscience.com



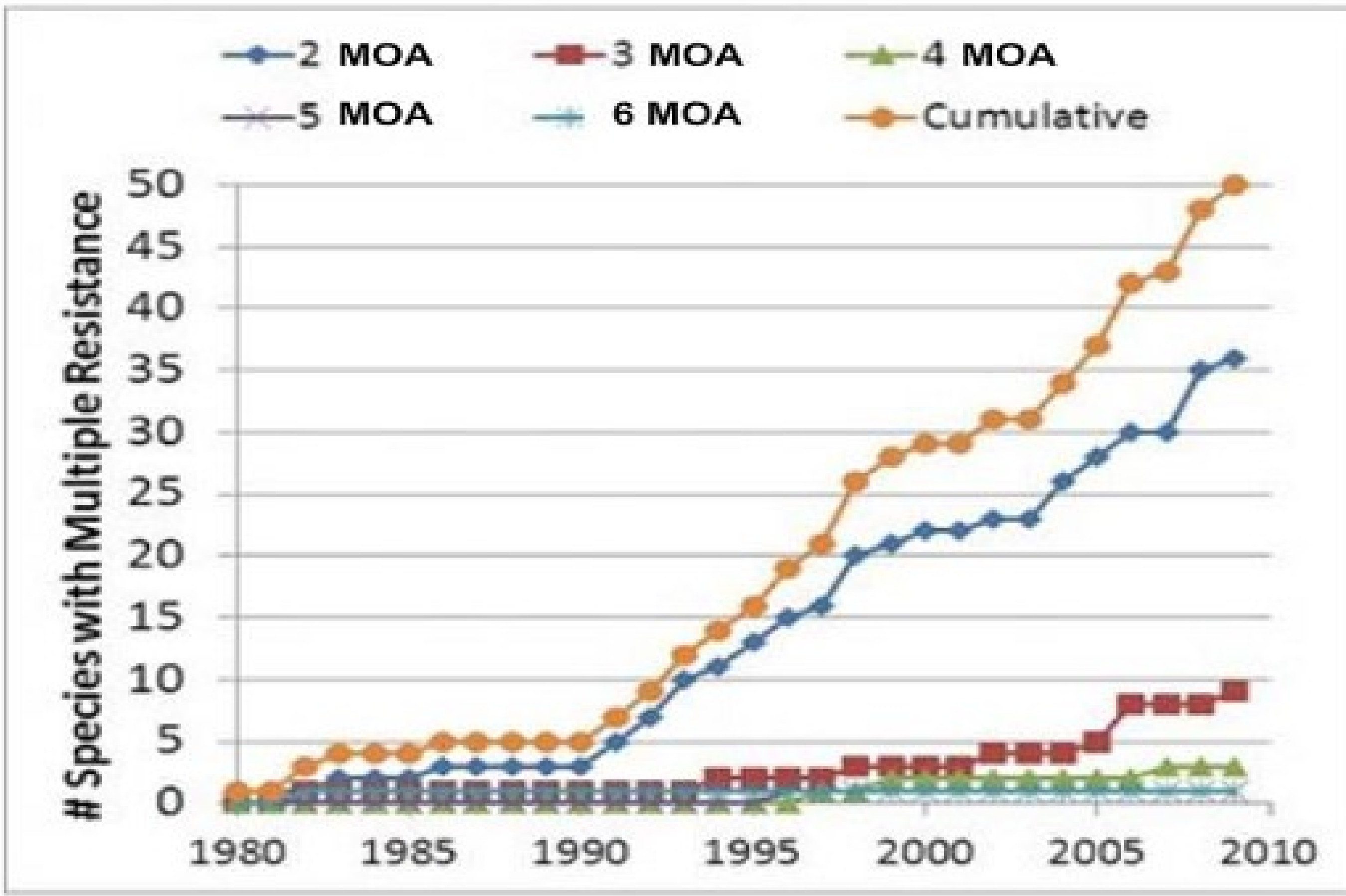
Source: Ian Heap
http://www.weedscience.com

Roundup Ready Crop Acres



Source: USDA - ERS

Weeds with Resistance to Multiple Modes of Action



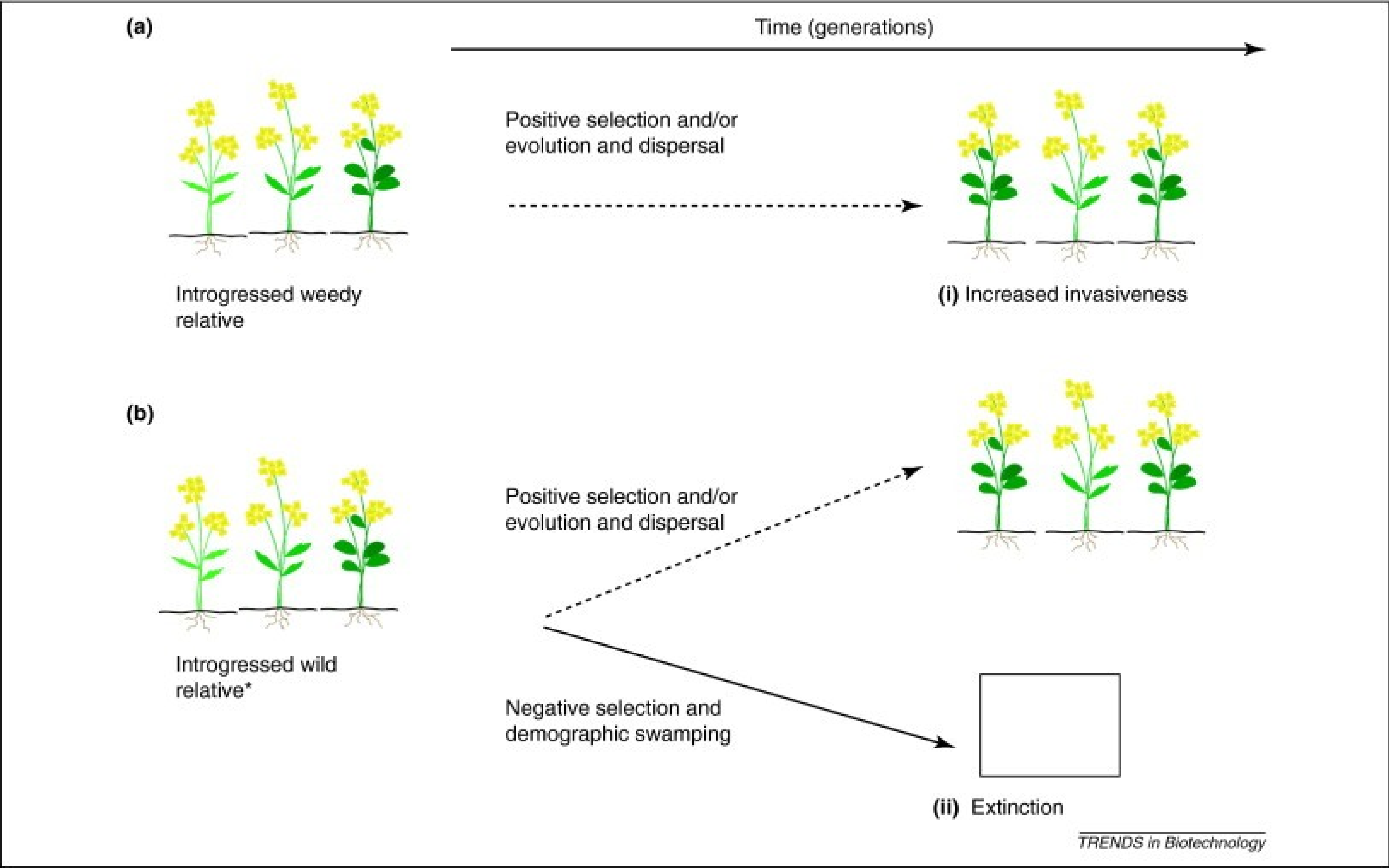
Source www.wssa.net

How does resistance occur?

- Spontaneous mutations

- Do herbicides cause mutations?
  - NO!! Herbicides provide selection pressure that finds the mutation after it has occurred.

Natural selection in Mutations



Increasing Selection Pressure

1. Multiple applications in one season.
2. Use for consecutive growing seasons
3. No other control options (cultivation, etc.)

Why are plants resistant to herbicides?/ Mechanism of resistance in weeds

- Altered herbicide binding site

  Extremely high levels of resistance

- Improved herbicide metabolism

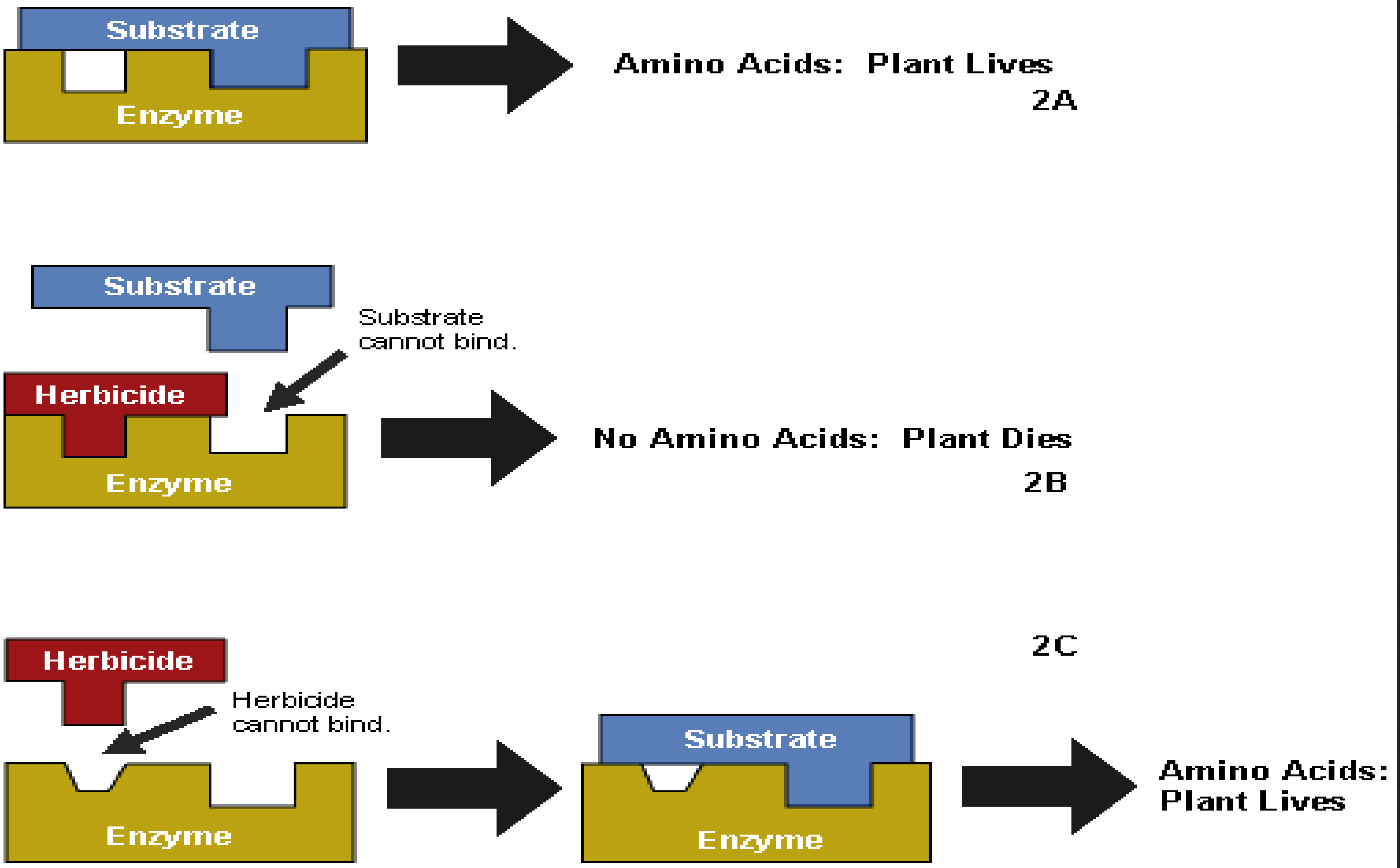
  Slight resistance

- Sequestration (Movement of herbicide is impeded)

Altered herbicide binding site

- **Genetic mutations** within a herbicide site of action can prevent complete herbicide interaction with binding sites, allowing the target-site protein to remain functional.
- The incomplete inhibition of the altered site of action may result in little to no observed plant injury.
- Where the herbicide has such little inhibitory effect on the site of action, plants may survive greater than 10 times the normal herbicide rate (considered high-level resistance).
- Mechanisms of action where high-level resistance is most often seen include **ACCase, ALS, and photosystem II inhibitors**.

Altered herbicide binding site in AA synthesis inhibitors



II. Improved herbicide metabolism

- Rapid degradation (Corn degrades atrazine)
- This type of resistance is **more complex** than altered site-of-action type resistance because it involves several plant processes.
- Plants with altered metabolism resistance can **degrade several unrelated herbicides of different modes of action** through multiple genes controlling metabolic processes.
- Plant injury may occur because plants cannot rapidly degrade absorbed herbicide, causing this mechanism to be considered low-level resistance.
- Increasing the herbicide rate to smaller plants may control more plants.

Examples:

- ✓ Ryegrass resistant to Acetyl coenzyme A carboxylase, Acetolactate synthase , and photosystem II inhibitors.
- ✓ Velvetleaf resistant to atrazine.
- ✓ In simazine resistance, the herbicide is acted upon by cytochrome P-450 monoxygenase enzyme and converted to herbicidally inactive de-ethyl simazine and di-de-ethyl simazine
- ✓ Simazine resistance in *Lolium rigidum*

III. Sequestration

- **Movement of herbicide is impeded**, moved away from its target site, or moved to a location where it is ineffective.
- This may be at the whole-plant or cellular level.
- Nearly all plants with this type of resistance are injured shortly after the herbicide application because the herbicide can not be moved away from the site of action fast enough and for a long enough time.
- Herbicide sequestration is considered **low-level resistance** because increasing rates applied to smaller plants increases mortality.

Examples:

Glyphosate-resistant biotypes of horseweed, ryegrass, common and giant ragweed.

Factors controlling the development of resistant weeds

- ✓ Weed Characters
- ✓ Herbicide characteristics
- ✓ Cultural practices

I. Weed Characters

- ✓ Annual growth habit
- ✓ High seed production.
- ✓ Relatively rapid turnover of the seed bank due to high percentage of seed germination each year (i.e., little seed dormancy).
- ✓ Several reproductive generations per growing season.
- ✓ Extreme susceptibility to a particular herbicide.
- ✓ High frequency of resistant gene(s), (e.g. Lolium rigidum).

II. Herbicide characteristics

- ✓ A single site of action
- ✓ Broad spectrum control.
- ✓ Long residual activity in the soil.

III. Cultural practices

- Shift away from multi crop rotations towards mono cropping.
- Little or no cultivation or tillage for weed control or no elimination of weeds that escape herbicide control.
- Continuous or repeated use of a single herbicide or several herbicides that have the same mode of action.
- High herbicide use rate relative to the amount needed for weed control.
- Orchard and vineyard systems.
- Roadsides

How to Prevent or Delay Herbicide Resistance

- ✓ Herbicide rotation
- ✓ Crop rotation
- ✓ Monitoring after herbicide application
- ✓ Non-chemical control techniques
- ✓ Short-residual herbicides
- ✓ Certified seed
- ✓ Clean equipment

How to Manage Herbicide-Resistant Weeds

- ✓ Herbicide rotation
- ✓ Fallow tillage
- ✓ Close cultivation
- ✓ Prevention of weed seed spread through use of clean equipment.
- ✓ Monitoring the initial evolution of resistance by recognizing patterns of weed escapes typical of resistant plants.
- ✓ Control of weeds suspected of herbicide resistance before they can produce seed.

Conditions leading to a fast development of resistance

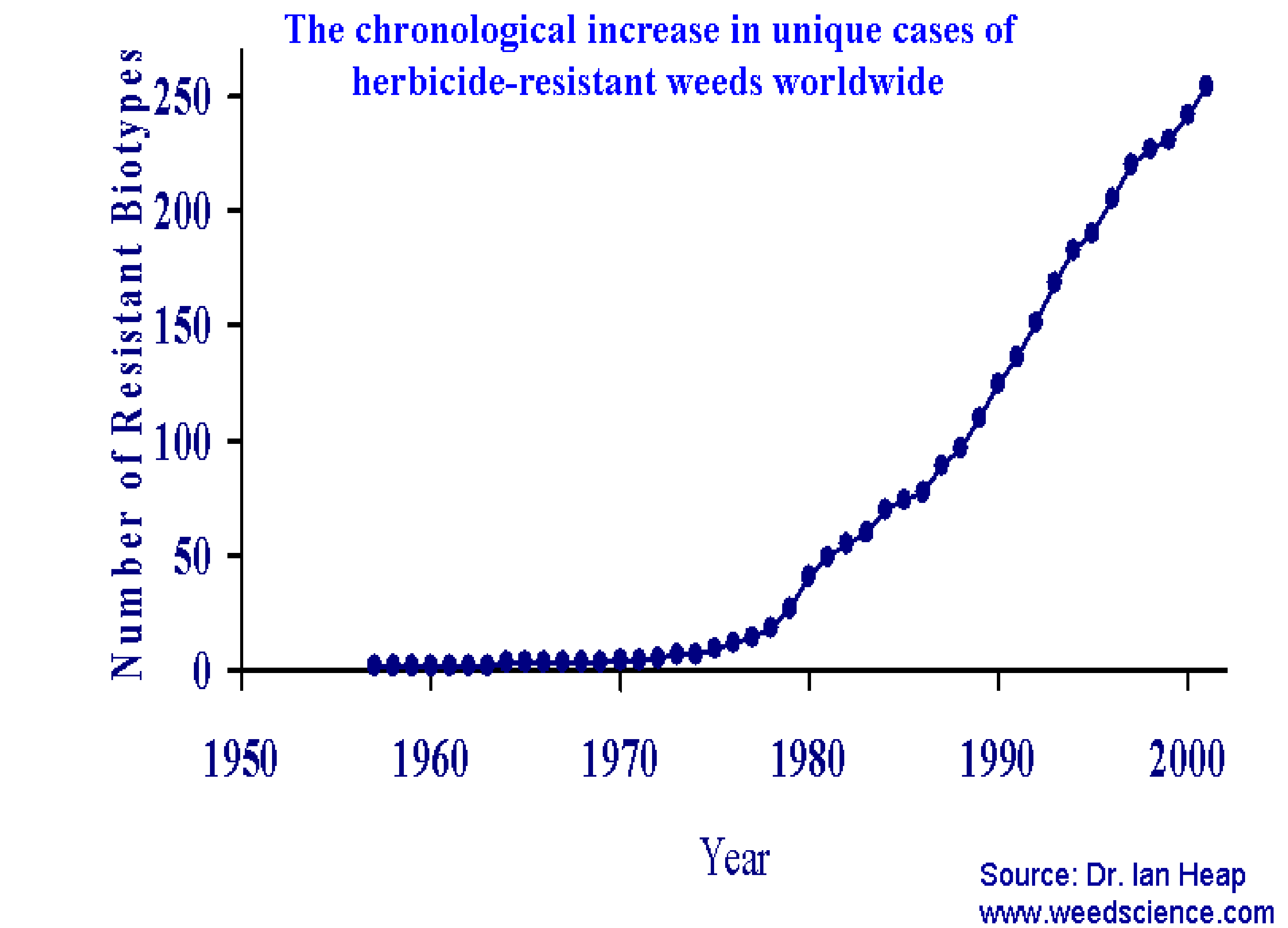
- High persistence of the herbicide
- Low herbicide and crop rotation
- High initial frequency of herbicide resistance gene
- High mutation frequency of the resistance gene
- High selection pressure of the herbicide
- Single mode of action
- Gene flow from herbicide resistant crops to weedy relatives

(sources: Thill DC. 1996 Managing the Spread of Herbicide Resistance. In: Duke SO. (ed.) HerbicideResistant Crops: 331-337. New York)

Conditions under which the portion of resistant biotypes quickly increases

- Short-living seeds, few seeds in soil
- High amounts of pollen distribution over long distances

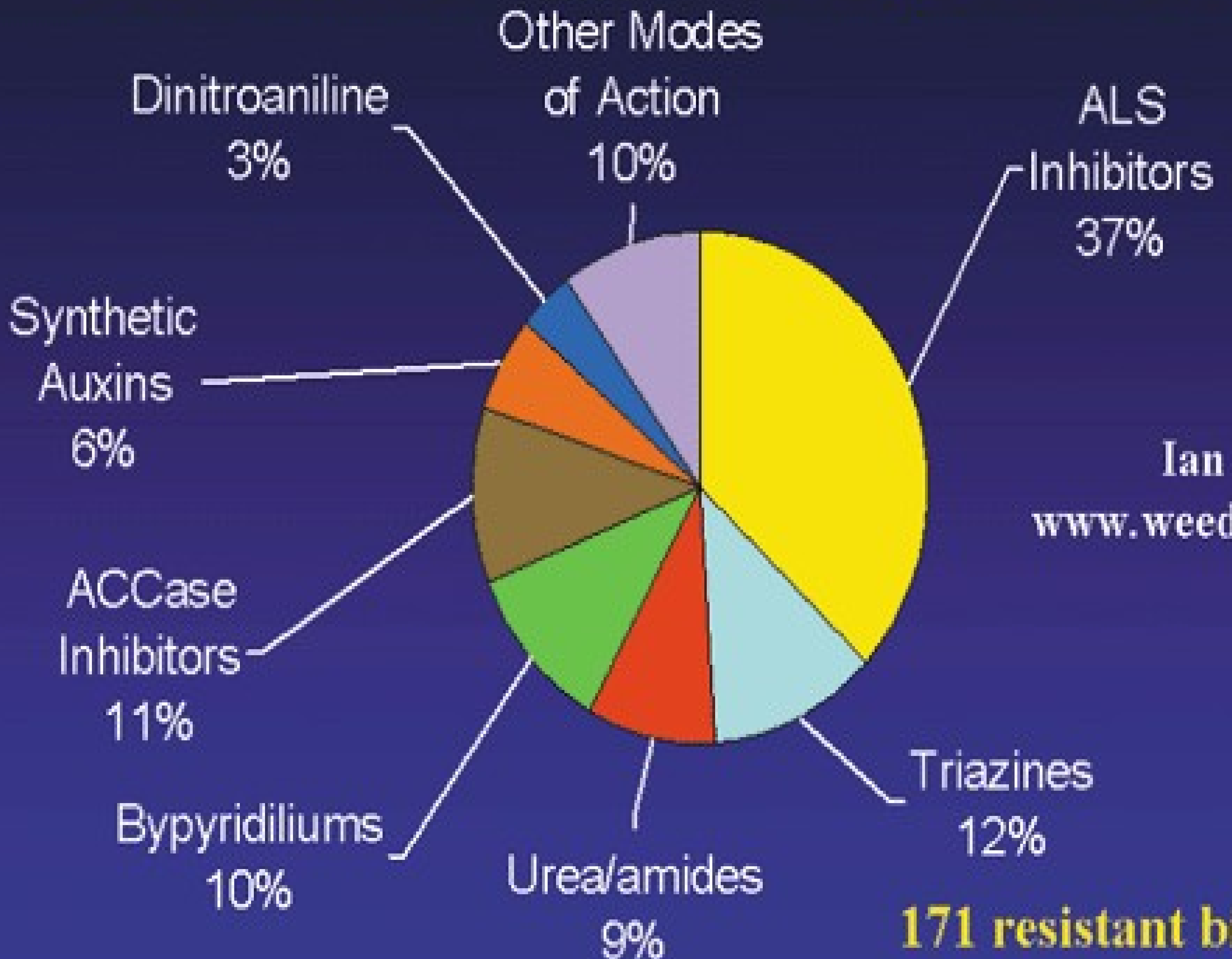
(sources: Thill DC. 1996 Managing the Spread of Herbicide Resistance. In: Duke SO. (ed.) HerbicideResistant Crops: 331-337. New York)


The chronological increase in unique cases of herbicide-resistant weeds worldwide
Number of Resistant Biotypes
250
200
150
100
50
0
1950
1960
1970
1980
1990
2000
Year
Source: Dr. Ian Heap
www.weedscience.com

Most Important Herbicide-Resistant Species

1.	Rigid Ryegrass	*Lolium rigidum*
2.	Wild Oat	*Avena fatua*
3.	Redroot Pigweed	*Amaranthus retroflexus*
4.	Common Lambsquarters	*Chenopodium album*
5.	Green Foxtail	*Setaria viridis*
6.	Barnyardgrass	*Echinochloa crus-galli*
7.	Goosegrass	*Eleusine indica*
8.	Kochia	*Kochia scoparia*
9.	Horseweed	*Conyza canadensis*
10.	Smooth Pigweed	*Amaranthus hybridus*

Herbicide-Resistant Weeds (1984 to 1999)



Ian Heap
www.weedscience.com

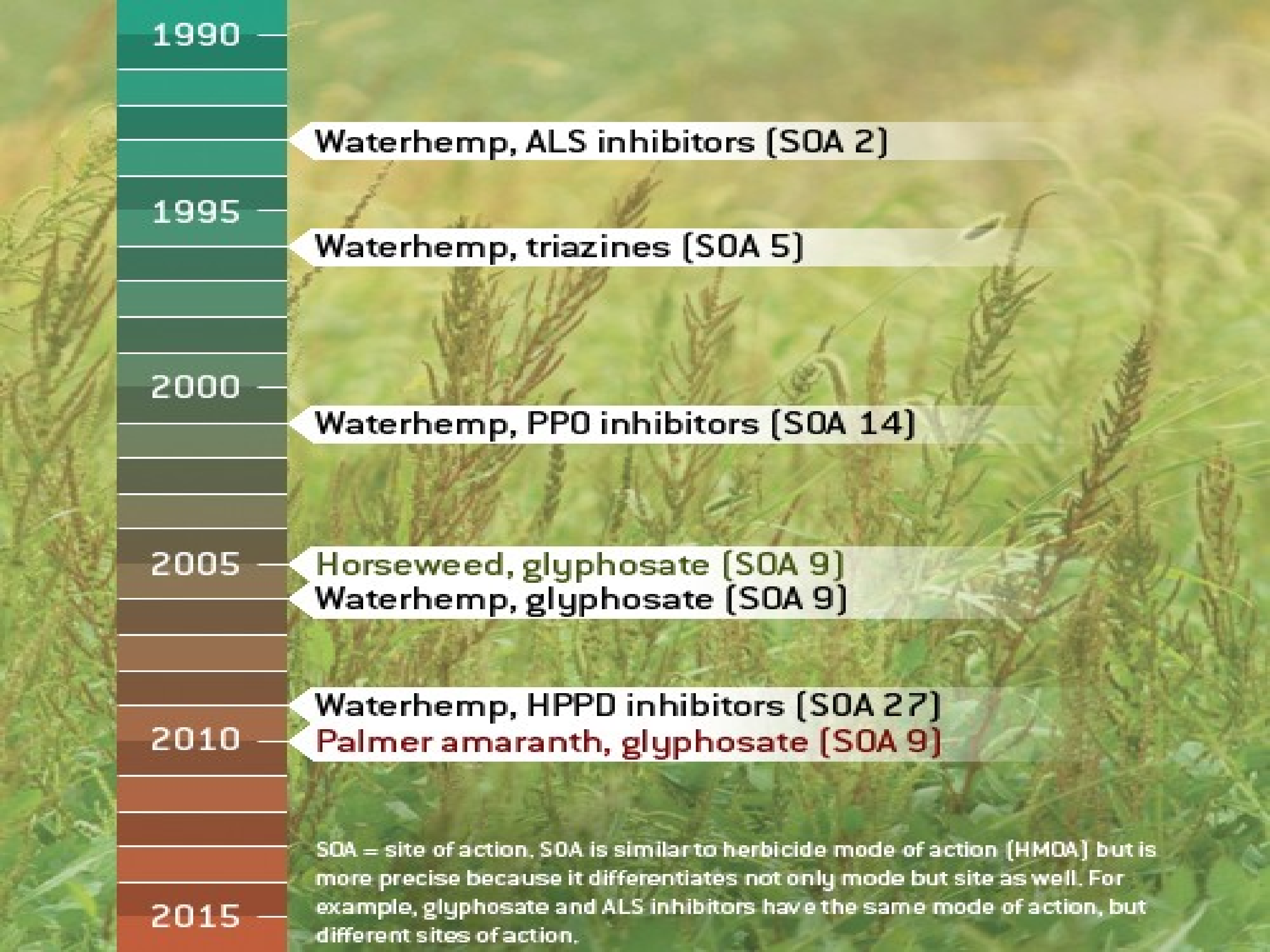
171 resistant biotypes

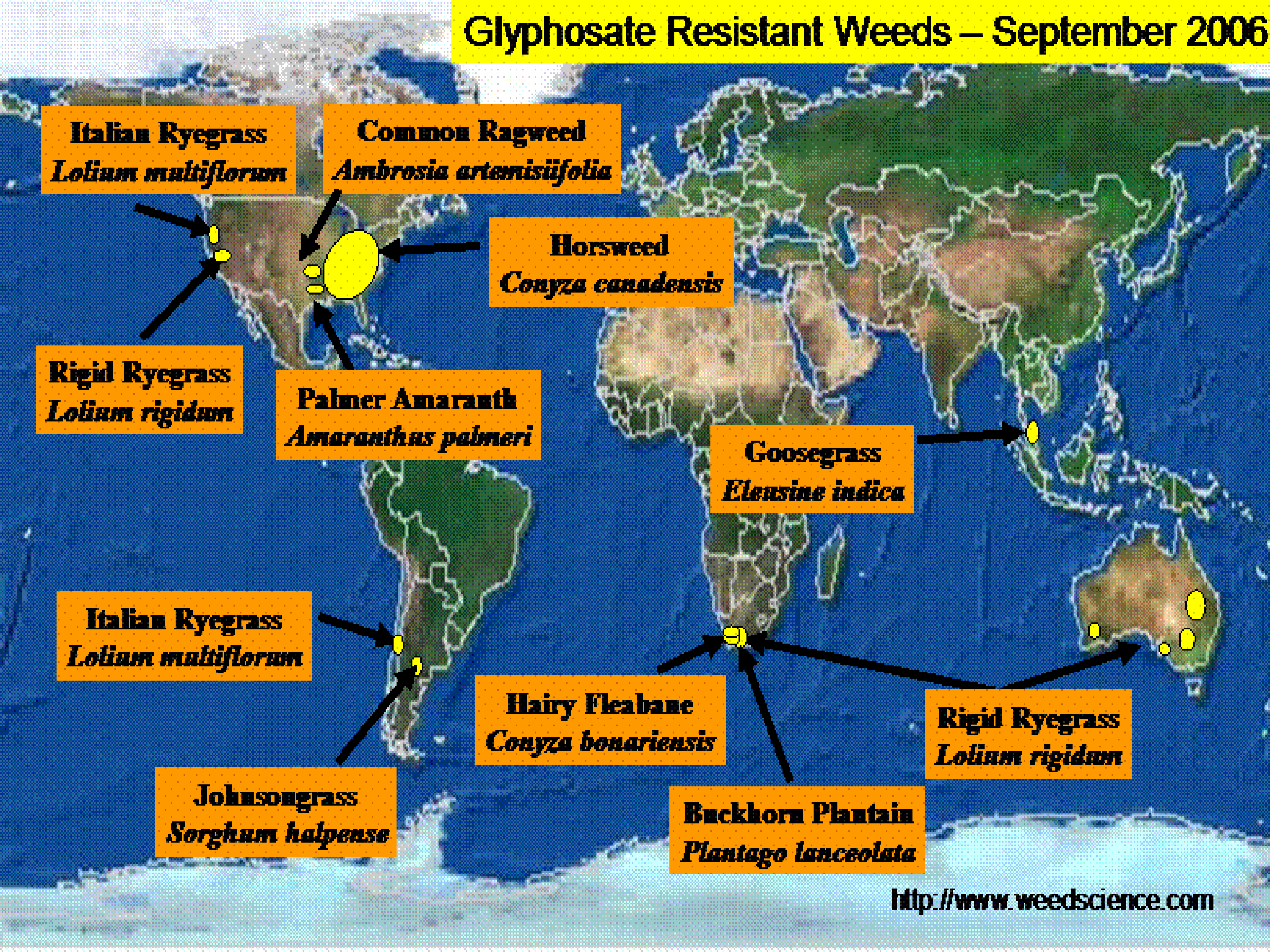
The effects of agronomic practices, weed characteristics and herbicide properties on the risk of developing herbicide-resistant weeds.

	--------------Risk of resistance-------------		
	Low	Intermediate	High
Weed control practices	Integration of cultural, mechanical, and chemical	Mechanical and chemical	Chemical
Crop rotation	Diversified rotation	Limited rotation	Monoculture
Weed abundance	Low	Intermediate	High
Weed susceptibility to a particular herbicide	Low	Intermediate	High
Weed seed longevity	Long	Intermediate	Short
Weed reproduction mechanism	Cross-pollination	Cross- and self-pollination	Self-pollination
Number of herbicide modes of action used per season	> 2	2	1
Number of site of action targeted by the herbicide	Multiple	Few	Single
Herbicide soil residual period	Short	Intermediate	Long

Table 2. Herbicide resistance risk factors

Agronomic factor	Least risk - - - - - -➤	Most risk
Cropping system	Good rotation	Continuous winter cereals
Cultivation system	Annual ploughing	Continuous non-ploughing
Control method	Cultural only	Herbicides only
Grass-weed herbicide use throughout the rotation	Different modes of action	Single mode of action
Weed infestation	Low	High
Resistance in vicinity	None	Common

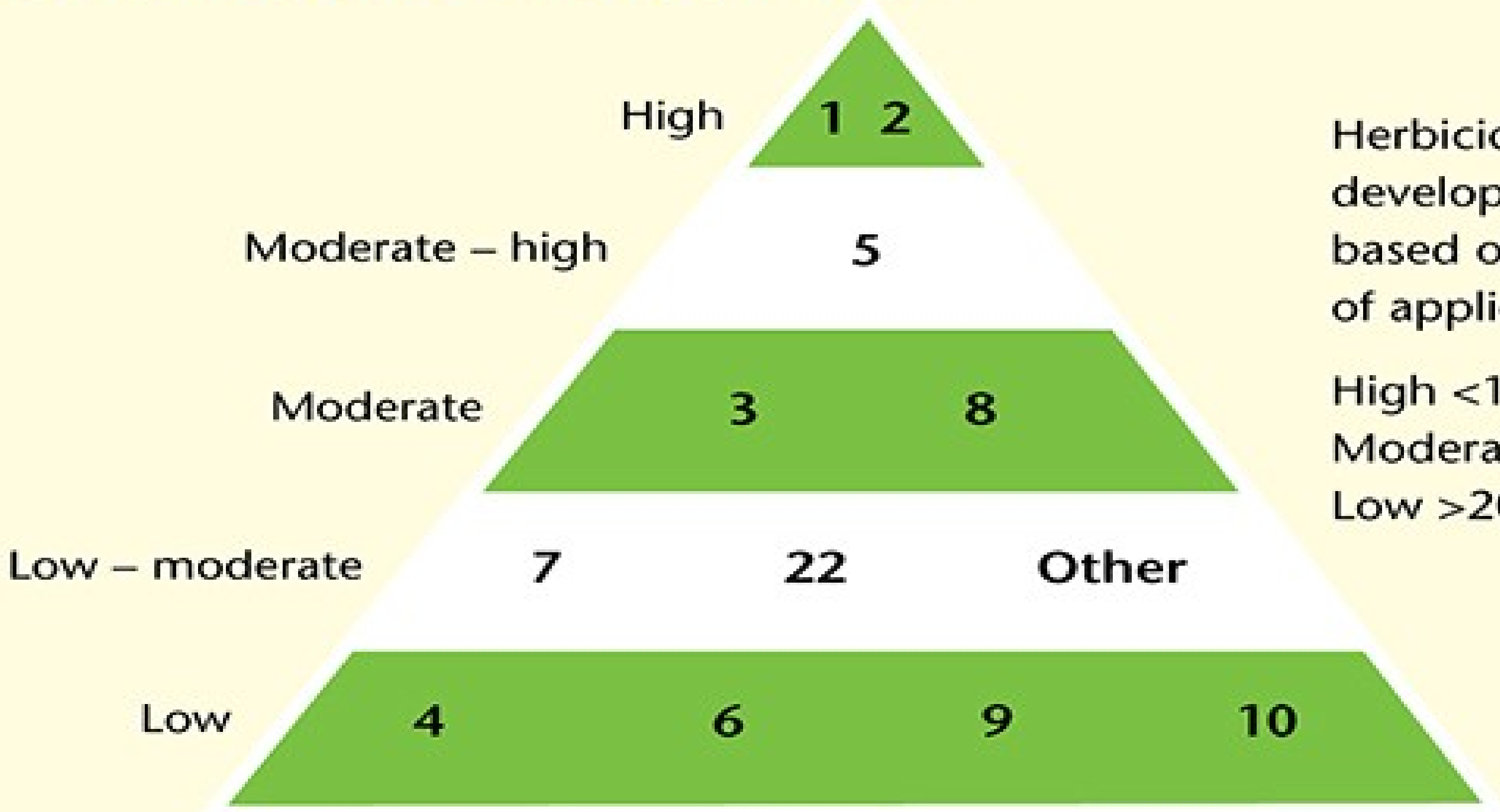

1990
Waterhemp, ALS inhibitors (SOA 2)
1995
Waterhemp, triazines (SOA 5)
2000
Waterhemp, PPO inhibitors (SOA 14)
2005
Horseweed, glyphosate (SOA 9)
Waterhemp, glyphosate (SOA 9)
Waterhemp, HPPD inhibitors (SOA 27)
2010
Palmer amaranth, glyphosate (SOA 9)
2015
SOA = site of action. SOA is similar to herbicide mode of action (HMOA) but is more precise because it differentiates not only mode but site as well. For example, glyphosate and ALS inhibitors have the same mode of action, but different sites of action.


Glyphosate Resistant Weeds – September 2006
Italian Ryegrass
Lolium multiflorum
Common Ragweed
Ambrosia artemisiifolia
Horsweed
Conyza canadensis
Rigid Ryegrass
Lolium rigidum
Palmer Amaranth
Amaranthus palmeri
Goosegrass
Eleusine indica
Italian Ryegrass
Lolium multiflorum
Hairy Fleabane
Conyza bonariensis
Rigid Ryegrass
Lolium rigidum
Johnsongrass
Sorghum halpense
Buckhorn Plantain
Plantago lanceolata
http://www.weedscience.com

Ten important herbicide mode of actions as per WSSA

Mode of action	WSSA group	No of resistant species
Acetyl coenzyme A carboxylase	Group 1	44
Acetolactate synthase	Group 2	142
Shoot inhibitors	Group 3	
PGR	Group 4	12
PS-II	Group 5	31
PSP	Group 9	72
PS-I	Group 22	25
Glutamine Synthetase	Group 10	29
Protoporphyrinogen Oxidase	Group 14	2
Cellulose inhibitors	Group 20/29	6

CLASSIFICATION OF HERBICIDE GROUP NUMBERS BY RISK OF SELECTION FOR WEED RESISTANCE



Herbicide resistance development risk based on number of applications:

High <10
Moderate 11 – 20
Low >20

Adapted from Beckle, H.J., 2006 Herbicide Resistant Weeds: Management Tactics & Practices Weed Technology Vol. 20 Issue 3 (July-September) pp. 793-814
('Other': all other herbicide groups that pose a low or moderate risk)

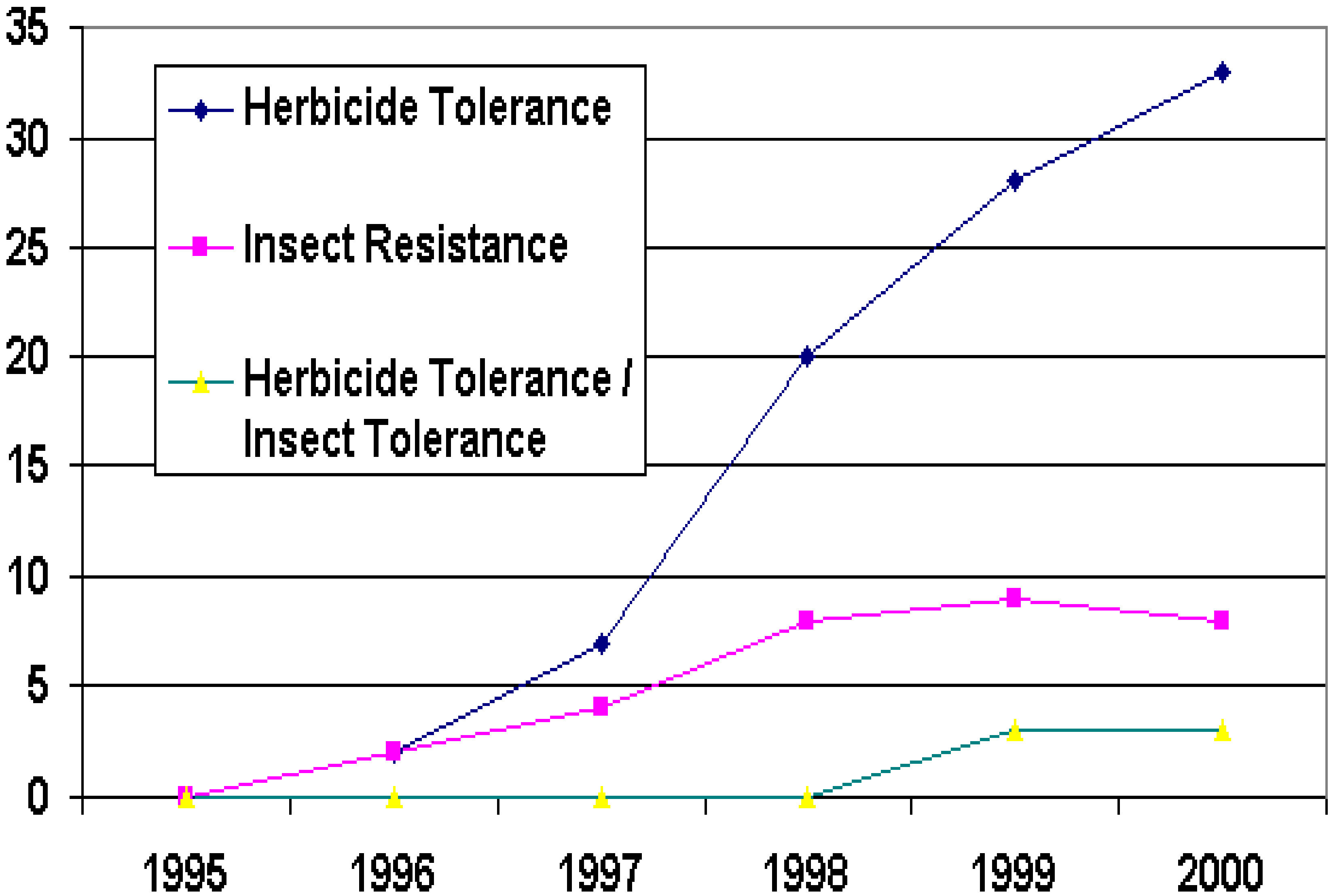
Group1 – ACCase inhibitors;
Group2 – ALS/AHAS inhibitors;
Group3 – Microtubule assembly inhibitors;
Group4 – Synthetic auxins;
Group5 – PS-II inhibitors Site A;
Group7 – PS-II inhibitors Site B;
Group8 – Lipid synthesis inhibitors (not ACCase inhibition)
Group9 – Inhibitors of EPSP synthesis;
Group10 – Glutamine Synthetase
Group15 – Inhibitors of cell growth and division;
Group22 – Cell membrane disruptors.

Herbicide Resistance Crops

Order of weed control practises followed in history

- Physical control
- Mechanical control
- Chemical weed control
- Biological control

Biotic resistance development in cultivated crop



Approaches to manage resistance weeds

- **Design of specific chemicals** with broad selectivity for crops
- **Development of crop cultivars** with tolerance to already existing effective broad spectrum herbicide

Why there is a shift from herbicides for control of weeds to crops resistant to herbicides

- Lack selectivity in effective broad spectrum herbicides available
- Selectivity is mostly based on differential herbicide uptake between **weeds and crops**
- Controlled timing and site of application
- **Rapid detoxification** of the herbicide by the crop plants.
- **Reliance on natural selective processes** limits the effective use of potent herbicides
- Development of herbicide resistance in weeds
- Increasing **difficulties in discovering new chemicals**

Herbicide tolerance via genetic transformation can be conferred by

- Introduction of a gene(s) coding for an herbicide detoxifying enzyme(s).
- Introduction of gene(s) coding for a herbicide insensitive form of a normal functioning enzyme or over expression of the genes coding for a herbicide target enzyme such that the normal metabolic functioning is still achieved in the plant even though some of the enzyme is inhibited.
- Modification of the herbicide target enzyme in such a way that the herbicide molecule does not bind to it and.
- The more recently described engineering of active herbicide efflux from plant cells.

Resistance to photosystem-II inhibitors

- Herbicides targeting the PS-II electron transport cascade function by binding to the **Qb site of the D_1 protein** thereby are **blocking the binding of plastoquinone.**
- The herbicide bromoxynil is used selectively to control broadleaf weeds in most monocot crops. Apparently, these crops have a metabolic pathway that inactivates the herbicide.

- To extend the use of this herbicide in dicots a gene (*bxn*) coding for an active nitrilase enzyme was cloned from a strain of the soil bacterium, *Klebsiella ozaenae* (Stalker and Mcbride, 1987).
- Introduction of the ***bxn*** gene into cotton via *Agrobacterium* transformation produced plants that were able to hydrolyze bromoxynil and ioxynil into non-toxic compounds, thus conferring resistance (Stalker et al., 1996).
- Bromoxynil resistant canola was developed using the same system by Calgene Inc and Aventis Crop Sciences Inc (AgBios, GM database).

Engineering resistance to Glufosinate ammonium

- Glufosinate ammonium (GA) is a non-selective pro-herbicide that is converted by plants into the phytotoxin, **phosphinothricin** (PPT).
- The herbicide acts by inhibiting the essential ammonia assimilation enzyme, **glutamine synthetase** (GS).
- The strategy to develop glufosinate resistant crops was based on knowledge of where the herbicide was naturally produced.
- A glufosinate was discovered as an antibiotic (detoxification) produced by the fungus, ***Streptomyces hygroscopicus*** (Mase, 1984).
- ***Bar*** **(bialaphos resistance) gene**, which encodes a **phosphinothricin acetyl transferase (PAT)** that converts the herbicidal molecule to a **non-toxic acetylated form** (Thompson *et al*., 1987).
- Wohlleben *et al*., (1988) also isolated the gene from a related species, *S. viridochromogenes*, and used it to produce transgenic **phosphinothricin resistant tobacco**, **potato and tomato plants**.

- Since its discovery, the *bar/pat* gene system has been used to engineer glufosinate tolerance in many crops.
- Glufosinate tolerant maize, cotton, sugar beet, canola and soybeans have been approved for commercial production by the United States food and drug administration (FDA).

Glufosinate ammonium herbicide resistant crops & developers

S. No	Crop	Reference
1	Corn	Gordon-Kamm *et al*., 1990
2	Rice	Christou *et al*., 1991
3	Wheat	Vasil *et al*., 1992
4	Sugarbeet	D'Halluin *et al*., 1992
5	Oilseed rape and alfalfa	Cobb, 1992
6	Cotton	Keller *et al*., 1997
7	Lettuce	Mohapatra et al., 1999
8	Sugarcane	Falco *et al*., 2000
9	Cassava	Sarria *et al*., 2000
10	Dry beans	Aragao *et al*., 2002

Mulwa & Mwanza; 2006. Biotechnology approaches to developing herbicide tolerance/selectivity in crops; African Journal of Biotechnology Vol. 5 (5), pp. 396-404,

Engineering tolerance to growth regulator herbicides

- First class of chemical weed control agents to be commercially introduced for agricultural use in the 1940s.
- Most widely used herbicides today.
- Mimic hormonal activity (2, 4-D as auxin towards dicotyledonous plants)
- Degradation of 2, 4 - D by numerous soil bacteria contain plasmid-based gene/ protein cascades for its metabolism.
- The best characterized of the 2,4-D degrading bacteria is ***Ralstonia eutrophus*** (formerly *Alcaligenes eutrophus*).
- The gene *tfdA* for the first step of 2,4-D degradation pathway was isolated from the plasmid, pJP_4, of the bacterium (Streber *et al*., 1987) was shown to encode a 2,4-D dioxygenase, which degrades 2,4-D into the inactive compounds i.e., glyoxylate & 2,4-dichlorophenol.

- Transformation of tobacco with a plant expressible form of the *tfdA* gene conferred 10- fold resistance to the herbicide compared to non- transformed plants.
- The *tfdA* gene in cotton, produced plants that were tolerant to three times the field application rate of 2,4-D used in wheat, corn, sorghum and pasture crops (Bayley et al., 1992).
- **Cotton** varieties with tolerance to 2,4-D conferred by the *tfdA* gene have been developed and **released in Australia** (Llewellyn and Last, 1996).
- Recently, the *tfd*A gene has been incorporated into the genome of wine grapes (*Vitis* inter specific hybrid 'Chancellor') and found to confer resistance to up to 20-fold the rate of 2,4-D applied in corn (Mulwa, 2005).
- This work was carried out to minimize 2,4-D vapor-drift damage in grapes, a serious limiting factor to the establishment of viticulture in grain producing regions of the United States.
- Studies are also in progress on dicamba (volatile growth regulator) resistant gene from *Pseudomonas maltophilia* strain DI-6 were cloned and expressed in *E. coli* (University of Nebraska).
- Induction of dicamba tolerance in broadleaf crops in tomato & tobacco on progress.

Engineering tolerance to Glyphosate

- Glyphosate (Round up) is a very effective broad spectrum herbicide that inhibits the enzyme **5- enol pyruvyl shikimate phosphate (EPSP) synthase**

- Which is essential for **protein biosynthetic pathway** of the aromatic amino acids i.e., tryptophan, tyrosine & phenylalanine, which are essential for and also as precursors for hormones, lignins, and other protective compounds such as flavanoids and alkaloids.

- EPSP synthase uses **phosphoenol pyruvate (PEP)** and shikimate-3-phosphate as substrates to make EPSP.

- **Glyphosate competitively interferes with the binding of PEP to the active site of EPSP synthase.**

- Genetic engineering work to develop glyphosate resistant crops focused on three strategies:

    1. Overproduction of EPSP synthase
    2. Introduction of a metabolic detoxification gene
    3. Introduction of an altered EPSP synthase enzyme with decreased affinity for glyphosate.

Altered EPSP synthase:

- Glyphosate resistant transgenic tobacco with a modified EPSP synthase encoded by the ***aroA*** **gene** of ***Salmonella typhimurium*** in which an **amino acid substitution of a proline for serine** caused a decreased affinity for glyphosate without affecting the kinetics of the enzyme.
- EPSP synthase gene (*cp4*) was identified from *Agrobacterium* sp. strain **CP_4**, whose protein product had favorable glyphosate tolerance kinetic parameters. Eg: Soybeans (Padgette *et al*., 1995).
- Glyphosate resistance through metabolic detoxification has also been explored.

Metabolic detoxification

- Two glyphosate detoxification pathways involving a glyphosate oxido reductase gene (***gox***) are known in microbes.
- The first involves oxidative cleavage of the N-C bond to yield **aminomethyl- phosphonic acid** (AMPA), which is further metabolized into **inorganic phosphate**
- The second pathway involves the breaking of the C-P bond by a carbon-phosphorus lyase to yield sarcosine, which is further metabolized into non-toxic components.

- The **glyphosate oxidoreductase (*GOX*) gene** was cloned from ***Pseudomonas* sp. strain LBr** (Franz *et al*., 1997) and has been used alongside the ***cp4* gene** to confer glyphosate resistance in a number of commercially available crops including **soybeans, corn**, anola, and **cotton**.
- This system has formed the basis of the so-called **Roundup-Ready crops**.

Engineering tolerance to plant pigment biosynthesis inhibitors

- Chlorophylls and carotenoids are two essential pigment classes in plants.

- Herbicides target one of two enzymes: **protoporphyrinogen oxidase** (protox or PPO) in the chlorophyll/ heme biosynthetic pathway or **phytoene desaturase (PDS)** in the carotenoid biosynthetic pathway, respectively.

- PPO is the target enzyme for the peroxidizing class of herbicides, which includes di-phenyl ether (DPE) and cyclic imide herbicides.

- PPO catalyzes the oxidation of protoporphyrinogen-IX to protoporphyrin-IX.

- The inhibition of protox by DPE herbicides leads to an abnormal accumulation of the tetrapyrole intermediate, protoporphyrin-IX, a photo-sensitizer that causes the generation of oxygen radicals (singlet oxygen).

- This light-dependent free radical production provokes lipid peroxidation in cellular membranes leading to plant death by excessive membrane and cellular constituent damage.
- PPO of *Bacillus subtilis* contains PPO gene, *hemY* is resistant to the herbicides.

- Tobacco resistant to oxyfluorfen - Hanson and Hederstedt, 1992
- Rice resistant to oxyfluorfen - Lee *et al*. 2000
- Corn (Mutant PPO gene) -Li & Nicholl,2005 (Acuron® technology (Holmberg,)

- **Carotenoid biosynthetic pathway** of plants, **phytoene desaturase** (PDS) is the target of several bleaching herbicides.
- PDS catalyzes the conversion of **phytoene to –caroten**
- Plants treated with these PDS inhibitor herbicides accumulate phytoene and suffer concurrent bleaching.

- PDS of ***Erwinia uredovora***, encoded by the ***crtl*** gene, was resistant to the bleaching herbicides.
- Transgenic tobacco with this gene yielded transgenic plants with strong multiple resistance to the herbicides norflurazon, fluridon, fluertamone, fluorochloridon and diflufenican
- This trait could provide **broad spectrum resistance** in incorporated in to more economically viable crops.

Engineering resistance to acetolactate synthase (ALS) inhibitors

- ALS is a **central enzyme** in the biosynthesis of the branched chain **amino acids** leucine, isoleucine & valine in plants.
- Herbicides targeting this enzyme belong to four structurally distinct classes: sulfonylureas, imidazolinones, pyrimidine sulfonamides & pyrimidinyl salicylates.
- These herbicide classes offer **effective selective control of grass weeds**.
- ALS is the target that is reported to have the **highest incidence of developing resistance to herbicides.**
- A large number of mutations have been characterized; most of them are due to **single amino acid sequence changes** that do not affect the enzyme function but easily induce herbicide resistance in plants where they occur
- This has resulted in the **emergence of a great number of weeds with resistance** to the sulfonylurea and imidazolinone herbicides classes.
- **Oligonucleotide mediated plant gene transfer** has been used to incorporate sulfonylurea resistance into several commercially important crops including cotton (Rajasekaran *et al*., 1996), soybeans (Aragao *et al*., 2000), canola (Mike *et al*., 1990), rice (Li *et al*., 1992), & flux (McHughen, 1989).

- Oligonucleotide mediated gene manipulation utilizes a technique that has for long been used in DNA repair and gene therapy studies in mammalian systems.
- Oigonucleotide made up of a combination of DNA and RNA bases, with a 32-base section having nearly exact homology to the target sequence of the endogenous plant gene.
- In this case ALS, except that there was a **single base mismatch** at the point of the desired mutation.
- The chimeric oligonucleotide was then delivered into the target cells using microprojectile bombardment, where it aligned with the endogenous homologous sequence.
- This resulted in a direct change in the targeted gene.
- Specifically, the procedure resulted in a change from **Ser621** (coded by **AGT**) **to Asn621** (coded by **AAT**), which conferred **resistance to imazethapyr and imazapyr in the regenerated plants**.
- This method of transformation is **fast and more convenient way** of developing herbicide resistance in a wide variety of crops without going through the rigors and expense of making specific genetic constructs.

Engineering tolerance to mitotic disruptor herbicides

- These are cell division inhibitors (dinitroanilines, DNAs) by disrupting the process of mitosis in mersitematic tissues of seedlings.
- Herbicide **molecules bind to the tubulin** protein subunits, and inhibit tubulin polymerization into microtubules
- Microtubules are the major constituent of the spindle apparatus, their **inhibition interferes with the movement of chromosomes to the metaphase configuration** as well as the migration of daughter chromosomes to their respective poles at anaphase.
- As a result, cell wall formation fails to occur at telophase.
- With time in the prophase state the chromosomes coalesce in the middle of the cell and a nuclear envelop reforms, resulting in a **polyploid nucleus**.
- Because new cells are not being formed, growth eventually stops and death results at an early seedling stage of the plant.
- Efforts to engineer crop resistance to mitotic disruptor herbicides started with the over-expression of mutant - and -tubulin proteins in maize calli
- This resulted in transgenic maize calli with resistance to DNAs and in which virtually all the endogenous tubulin synthesis was replaced with the transgenic protein.
- However, further development into plants was not possible because the maize callus lines used were not regenerable.

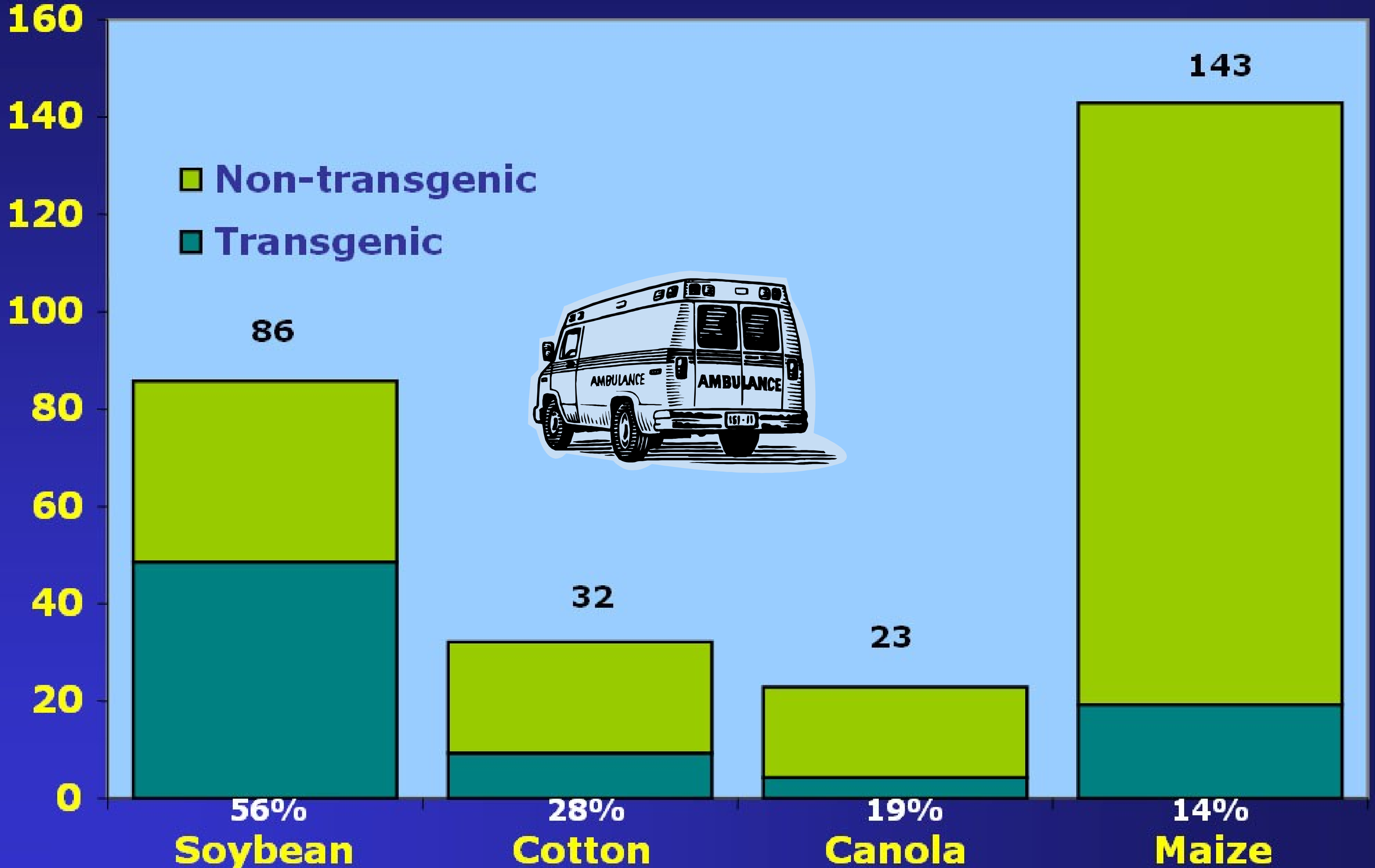
Disadvantages of transgenic herbicide resistance

- ✓ Mammalian toxicity
- ✓ Ecotoxicity (side effects on soil microorganisms and agricultural flora or fauna)
- ✓ Raising herbicide resistant weeds and volunteers
- ✓ Yield performance
- ✓ Single selection pressure and weed resistance
- ✓ Shifts in weed species
- ✓ Gene escape
- ✓ Gene flow and contamination of organic crops
- ✓ Drift and non-target movement

Advantages

- Facilitate low or no tillage
- Broader spectrum of weeds controlled
- Reduced crop injury
- Reduced herbicide carry-over
- Use of herbicides that are more environmentally friendly
- New mode of action for resistance management
- Crop management flexibility and simplicity

Global Adoption Rates (%) for Principal Biotech Crops (Million Hectares)



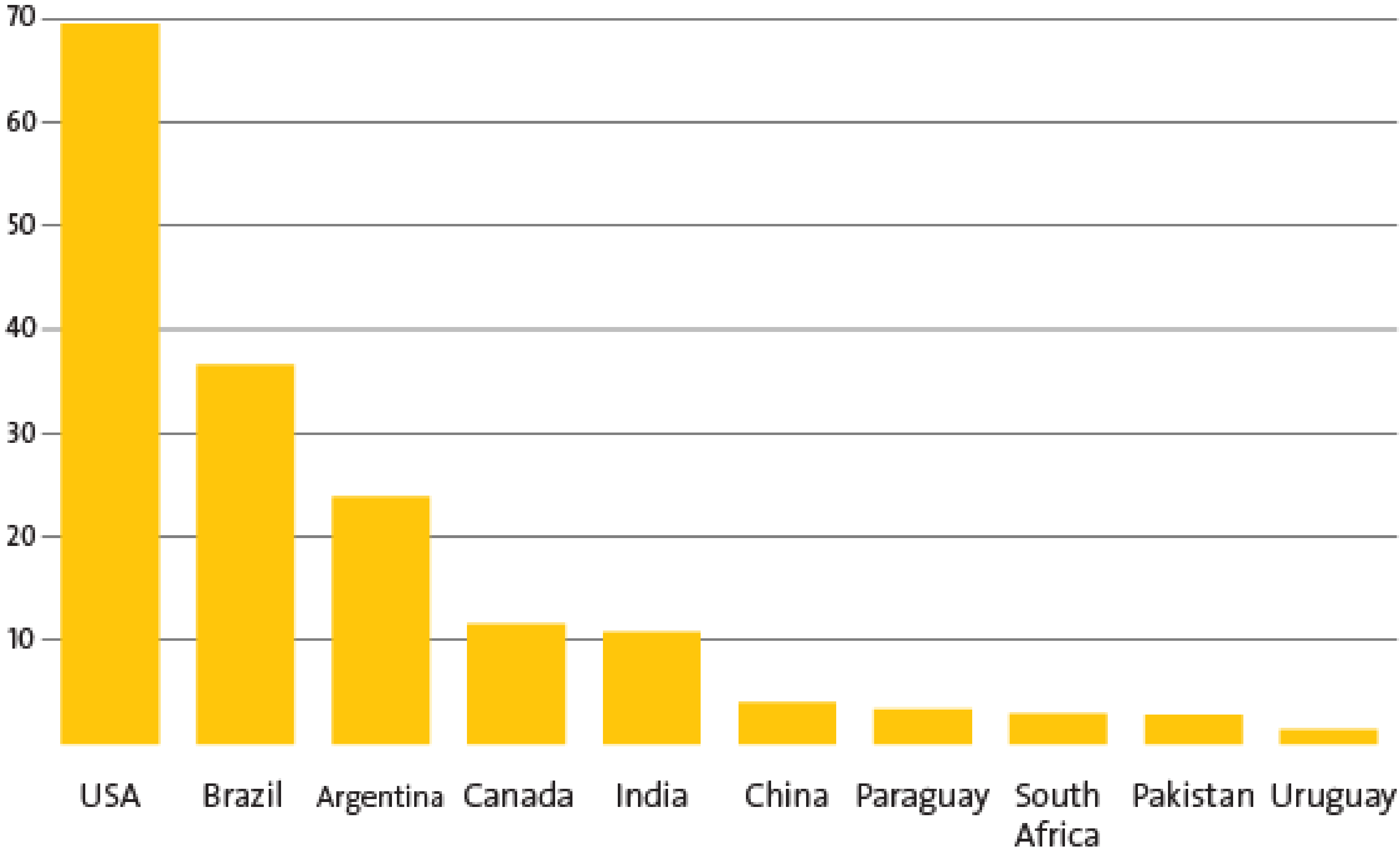
Source: Clive James, 2004

TABLE 1. Approved transgenic traits for U.S. crops

Trait	Examples
Herbicide tolerance	Bromoxynil, glufosinate, glyphosate, sulfonylurea
Insect resistance	Bt kurstaki, Bt tenebrionis
Virus resistance	Papaya ringspot virus, cucumber mosaic virus, zucchini yellow mosaic virus, watermelon mosaic virus, potato leaf roll virus, potato virus Y
Male sterility	Barnase/barstar
Modified ripening	ACC synthase, ACC deaminase, SAM hydrolase, polygalacturonase
Modified oils	High lauric, myristic, oleic acids

The World's Biggest GMO Lovers

Top GMO crop growing countries, in million hectares (2012)



Source: ISAAA

Mother Jones

Thanks!