

Glyphosate, Resistance, and Cancer: What is the Current Situation?

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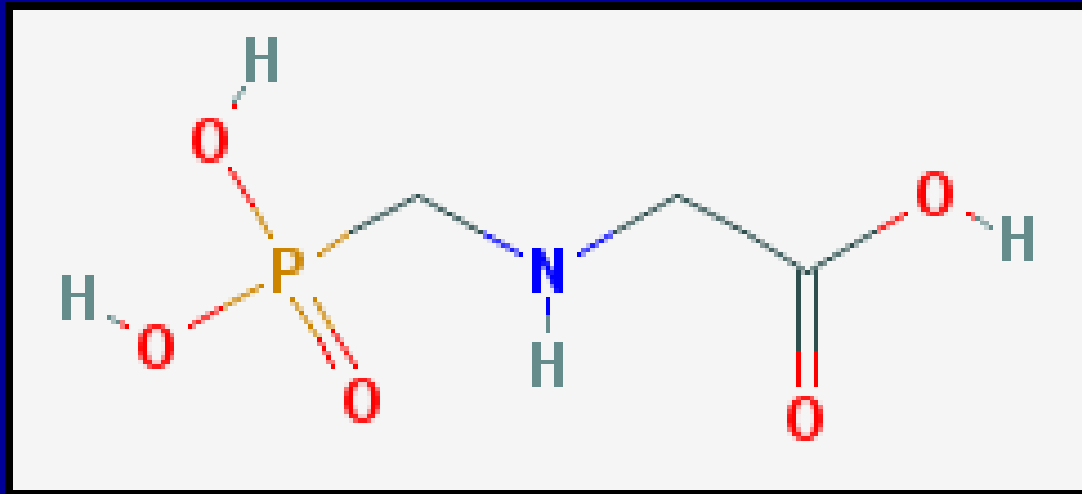
Glyphosate

- First registered in 1974 under the trade name Roundup
- Foliar-applied herbicide that is both phloem and xylem mobile
- Is tightly bound to phosphate sorption sites in soil, so soil activity is very rare
- Widely used in vineyards, orchards, fallow fields, and Roundup Ready crops

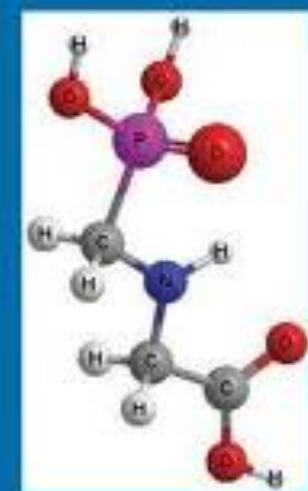


In This Session:

- Does Glyphosate Cause Cancer?
- Glyphosate Resistance (as time allows...)



GLYPHOSATE



Is Glyphosate Safe?

- Recent events have clouded the view!
- In particular, the finding by the **International Agency for Research on Cancer** (IARC, an agency within the World Health Organization) that glyphosate is a **Group 2A Carcinogen** was announced in March, 2015
- *Many thanks to Dr. Len Ritter, Professor Emeritus of Toxicology with University of Guelph, for much of the following timeline information*

Pesticide Registrations

- In the US and Canada, pesticides must undergo a reregistration process about every **15 years**
 - Registrants must **address all concerns** brought out in the science, especially regarding toxicological effects
- The last re-registration of glyphosate was in 1993, so the process for this herbicide began again a few years ago
 - **Canada's PMRA** (equivalent to US EPA) released its finding in **April, 2015**
 - **EPA's finding** was due December, 2015 but it has been backed off until this December (?)

Enter the IARC

- The IARC was born in 1965 in effort to “better understand the **occurrence, natural history, causes,** and **prevention** of cancer”
- **March, 2015:** Glyphosate were classified as “probably carcinogenic to humans (Group 2A)”
- What does this mean?

The IARC Selects Agents for Review Based on Two Criteria:

- The evidence of **human exposure**
- There is some **evidence or suspicion** of carcinogenicity

- Risk = Toxicity x Exposure
 - So cancer risk is based on the **inherent toxicity** of the agent and a **human's exposure** to that agent (frequency, duration, and intensity)

The IARC's Rating Scale

- Group 4: Probably not carcinogenic to humans (1 agent)
- Group 3: Not classifiable as to its carcinogenicity to humans (503 agents)
- Group 2B: Possibly carcinogenic to humans (287 agents)
- Group 2A: Probably carcinogenic to humans (73 agents)
- Group 1: Carcinogenic to humans (116 agents)



WHAT DOES
“PROBABLY
CAUSES CANCER”
ACTUALLY MEAN?

What Does The Record Say?

- 1993: EPA reregistered glyphosate
 - “Based on the results of its reregistration review, EPA has concluded that all registered uses of glyphosate are eligible for reregistration.”
 - “The Agency has classified glyphosate as a Group E carcinogen (signifies evidence of noncarcinogenicity in humans).”

An Updated Review of Glyphosate at the Advent of Roundup Ready

- Comprehensive review of glyphosate was conducted by Williams et al. (1998-2000)
 - Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient glyphosate for humans. *Regulatory Toxicology and Pharmacology* 31:117-165
 - “Multiple lifetime feeding studies **have failed to demonstrate any tumorigenic potential** for glyphosate. Accordingly, it was concluded that **glyphosate is noncarcinogenic.**”
 - Further, “It was concluded that, under present and expected conditions of use, **Roundup herbicide does not pose a health risk to humans.**”

WHO's Other Arm:

Joint Meeting on Pesticide Residues, 2004

- “Administration of glyphosate for two years produced **no evidence of a carcinogenic response** to treatment in rats. The NOAEL was 6000 ppm (= 361 mg/kg bw per day).”
 - “**No statistically significant increases** in the incidence of any **tumours**, either benign and malignant, in either sex when compared with the control groups.”

The Agricultural Health Study

- Joint project by EPA, National Cancer Institute, National Institute of Environmental Health Sciences, National Institute for Occupational Safety and Health
 - Since 1993, over 89,000 farmers and spouses in IA and NC have participated
- DeRoos et al. 2005. Cancer incidence among glyphosate-exposed pesticide applicators in the AHS. *Environmental Health Perspectives* 113:49-54
 - “Glyphosate exposure **was not associated with cancer** incidence overall or with most of the cancer subtypes we studied. There was a **suggested association with multiple myeloma** incidence that should be followed up as more cases occur in the AHS.”

Another Review, 2012

- Mink et al. Epidemiologic studies of glyphosate and cancer: a review. *Regulatory Toxicology and Pharmacology* 63:440-452
 - “To examine potential cancer risks in humans, we reviewed the epidemiologic literature to evaluate whether exposure to glyphosate is associated causally with cancer risk in humans. We also reviewed relevant methodological and biomonitoring studies of glyphosate. **Seven cohort studies and fourteen case control studies** examined the association between glyphosate and one or more cancer outcomes.”

The Mink et al. Review

- “Our review of the currently available epidemiologic literature on glyphosate and cancer found **no evidence** of a consistent pattern of positive associations that would be indicative of a **causal relationship between any site-specific cancer and exposure to glyphosate.**”

Let's Get Back To EPA

- 2013: “Based on the data summarized in Unit III.A,” *(the Mink et al. review)* “EPA has concluded that glyphosate does not pose a cancer risk to humans. Therefore, a dietary exposure assessment for the purpose of assessing cancer risk is unnecessary.”

Do You Remember DeRoos' Myeloma Question in 2005?

- Sorohan. 2015. Multiple myeloma and glyphosate use: a re-analysis of US Agricultural Health Study (AHS) Data. International Journal of Environmental Research and Public Health 12:1548-1559
 - “There were **no statistically significant trends for multiple myeloma risks** in relation to reported cumulative days (or intensity weighted days) of glyphosate use. The **doubling of risk reported previously** arose from the use of an **unrepresentative restricted dataset** and analyses of the full dataset provides **no convincing evidence** in the AHS for a link between multiple myeloma risk and glyphosate use.”

Germany Says: Re-register Glyphosate

- January, 2015: Germany, on behalf of the European Union, issued a renewal assessment report for glyphosate
 - “Classification and labeling for carcinogenicity is not warranted. This is based on a large number of long-term studies in rats (that) did not reveal any evidence of carcinogenicity. In the mouse, a higher incidence of malignant lymphoma was observed in one out of five carcinogenicity studies at an exaggerated dose level in a strain with high background incidence of this tumor type.”
 - “Epidemiological studies in the whole did not provide evidence of carcinogenicity in man.”

IARC's Report, March, 2015

- “For the herbicide glyphosate, there was **limited evidence of carcinogenicity** in humans for non-Hodgkin’s lymphoma.”
- “In addition, there is **convincing evidence** that glyphosate also can cause cancer in laboratory animals.”
- “The IARC Working Group that conducted the evaluation considered the significant findings from the USEPA report” (*EPA in 1985 had initially classified glyphosate as possibly carcinogenic to humans from data in a mouse study*) “and several more recent positive results in concluding that there is **sufficient evidence of carcinogenicity** in experimental animals.”

Germany Responds in April, 2015

- “In its recent evaluation, IARC...came to the conclusion that glyphosate should now be classified as a carcinogenic substance in Group 2A (probably carcinogenic to humans), based on ‘limited evidence’ in human experiments and ‘sufficient evidence’ in animal experiments. As the ‘Rapporteur Member State’ for the active substance glyphosate, the German Federal Institute for Risk Assessment (BfR) was responsible for the human health risk assessment and has assessed **glyphosate as noncarcinogenic.**”
- “The current report of BfR to the EU based on the evaluation of **over 30 epidemiological studies** came to the overall assessment that there is **no validated or significant relationship** between exposure to glyphosate and an increased risk of **non-Hodgkin lymphoma or other types of cancer.**”

Canada Weighs In, April, 2015

- “In consideration of the strength and limitations of the large body of information on glyphosate, which included multiple short and long term (lifetime) animal toxicity studies, numerous *in vivo* and *in vitro* genotoxicity assays, as well as the large body of epidemiological information, the overall weight of the evidence indicates that **glyphosate is unlikely to pose a human cancer risk.**”
- “An evaluation of available scientific information found that products containing glyphosate **do not present unacceptable risks to human health or the environment** when used according to label directions.”

One More Point by PMRA in Canada

- Remember Risk = Toxicity x Exposure?
- “The IARC recently assigned a hazard classification for glyphosate as ‘probably carcinogenic to humans.’ It is important to note that a **hazard classification is not a health risk assessment**. The level of exposure, which determines the actual risk, **was not taken into account by IARC**. Pesticides are registered in Canada only if the level of **exposure to Canadians does not cause any harmful effects, including cancer.**”
- “Products containing glyphosate are **unlikely to affect your health** when used according to label directions.”
- “When used according to proposed label directions, glyphosate products **do not pose an unacceptable risk to the environment**. Labelled risk-reduction measures mitigate potential risks posed by glyphosate formulations to non-target plants and freshwater/marine/estuarine organisms.”

Oh, and Remember That Other WHO Committee?

- Joint Meeting on Pesticide Registration, that in 2004 set NOAELs of 361 mg/kg bw per day (based on rat data)
- In June, 2015 pointed out that these **two committees have different functions**
 - JMPR to perform **risk assessment** for regulatory purpose
 - IARC to deal with **hazard identification**
- Proposed an *ad hoc* task force be formed to advise JMPR on how the studies IARC used to come to their opinion
 - In September, 2015 the Task Force found that **IARC had access to newer studies** than JMPR had available in 2004, and that JMPR must now **re-evaluate their finding of noncarcinogenicity**

European Food Safety Authority

- The **EFSA** (similar to the US Food and Drug Administration) released a report in November, 2015:
 - Concluded that glyphosate is **unlikely to be genotoxic** (i.e. damaging to DNA) or to **pose a carcinogenic threat to humans**
 - Glyphosate is **not proposed to be classified as carcinogenic** under the EU regulation for classification, labelling and packaging of chemical substances
 - In particular, all the Member State experts but one agreed that neither the **epidemiological data** (i.e. on humans) nor the evidence from **animal studies** demonstrated causality between exposure to glyphosate and the development of cancer in humans
 - EFSC set the acceptable operator exposure level at **0.1 mg/kg bw per day** and an acceptable daily intake for consumers was set in line with the ARfD (the German report) at **0.5 mg/kg bw per day**

What About The Surfactant?

- The EFSA also reported on the potential “genotoxicity, long-term toxicity, carcinogenicity, reproductive/developmental toxicity, and endocrine disrupting potential” of the polyethoxylated tallowamine (POEA) surfactant in Roundup formulations
- The group cited no available information on the residues in plants and livestock and concluded that “the available data are insufficient to perform a risk assessment” for the surfactant

Fast Forward to The Present

- Europe's **Commission for Health and Food Safety** had taken up the glyphosate re-registration issue, but could not come to consensus
- Recommended:
 - Continued use of glyphosate until **December, 2017**
 - **Removal of POEA from all herbicide formulations** until full risk assessment is possible
 - Pay “particular attention to **protection of groundwater** in vulnerable areas, in particular with respect to noncrop uses”
- In July, 2016, EU member states voted to accept these recommendations (22 in favor, 6 abstentions)

Bottom Line For Now

- IARC's assessment stands in sharp contrast to global assessment of the lack of carcinogenicity by glyphosate
- EPA will release its reregistration decision soon (?), but has signaled that it stands in agreement with the EU (Germany) and Canada in assessing glyphosate as being noncarcinogenic to humans

Herbicide Tolerance

- **Herbicide tolerance**: the inherent ability of a species to survive and reproduce after herbicide treatment; implies no selection or genetic manipulation to make the plant tolerant
 - “We’ve never gotten dependable control of this weed with this herbicide...”
 - *eg. grasses are not controlled by 2,4-D; thus it is widely used for selective control of broadleaf weeds in turf and cereals*

Herbicide Resistance

- **Herbicide resistance**: the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type
 - “We used to be able to control this weed with this herbicide, but it doesn’t work as well anymore...”
 - *eg. ALS inhibitors provided excellent control of weeds in PNW cereals for a few years after introduction, but resistant biotypes soon were reported*

Weed Population Shifts

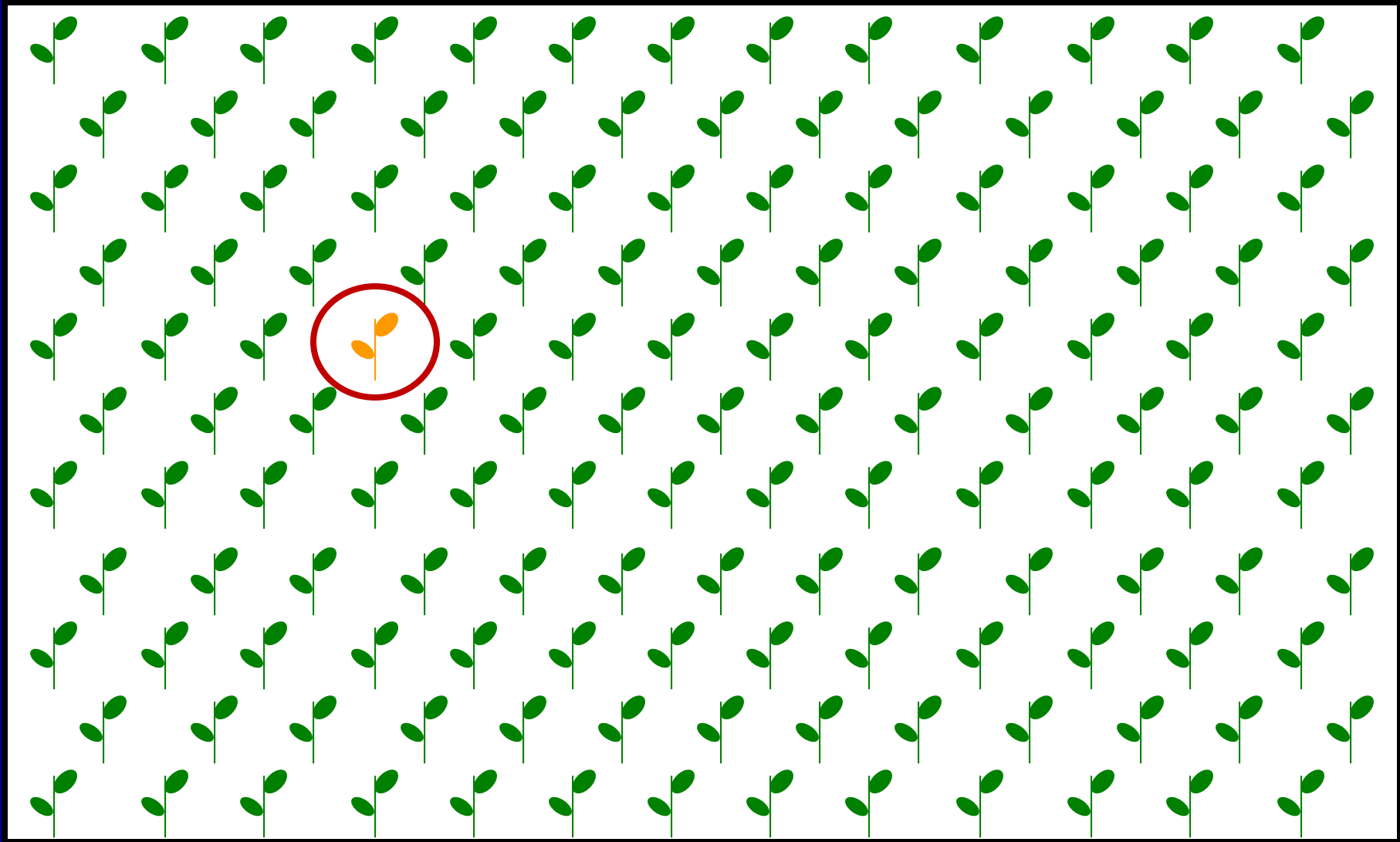
- Weed populations usually consist of a **mixture of species**
 - Same species are not always present in a field at all times
 - Relative proportion of individual species is **dynamic**, varying over time in response to management practices
- Repeated use of **any single control tactic** can lead to a weed population dominated by the species **not controlled by that practice**
 - Can occur through tolerance, resistance, or avoidance

Weed Shift Examples

- 2,4-D introduced in cereals in the 1950's
 - Shift from mostly **broadleaf weeds** to **grasses**
- Mowing or flaming in orchards
 - Shift to more **grasses or low-growing perennials**
- Perennial crops vs. annual crops
 - Shift to **perennials, biennials, and plants that don't tolerate disturbance**
- Selective herbicides
 - Shift to **tolerant or resistant weeds**

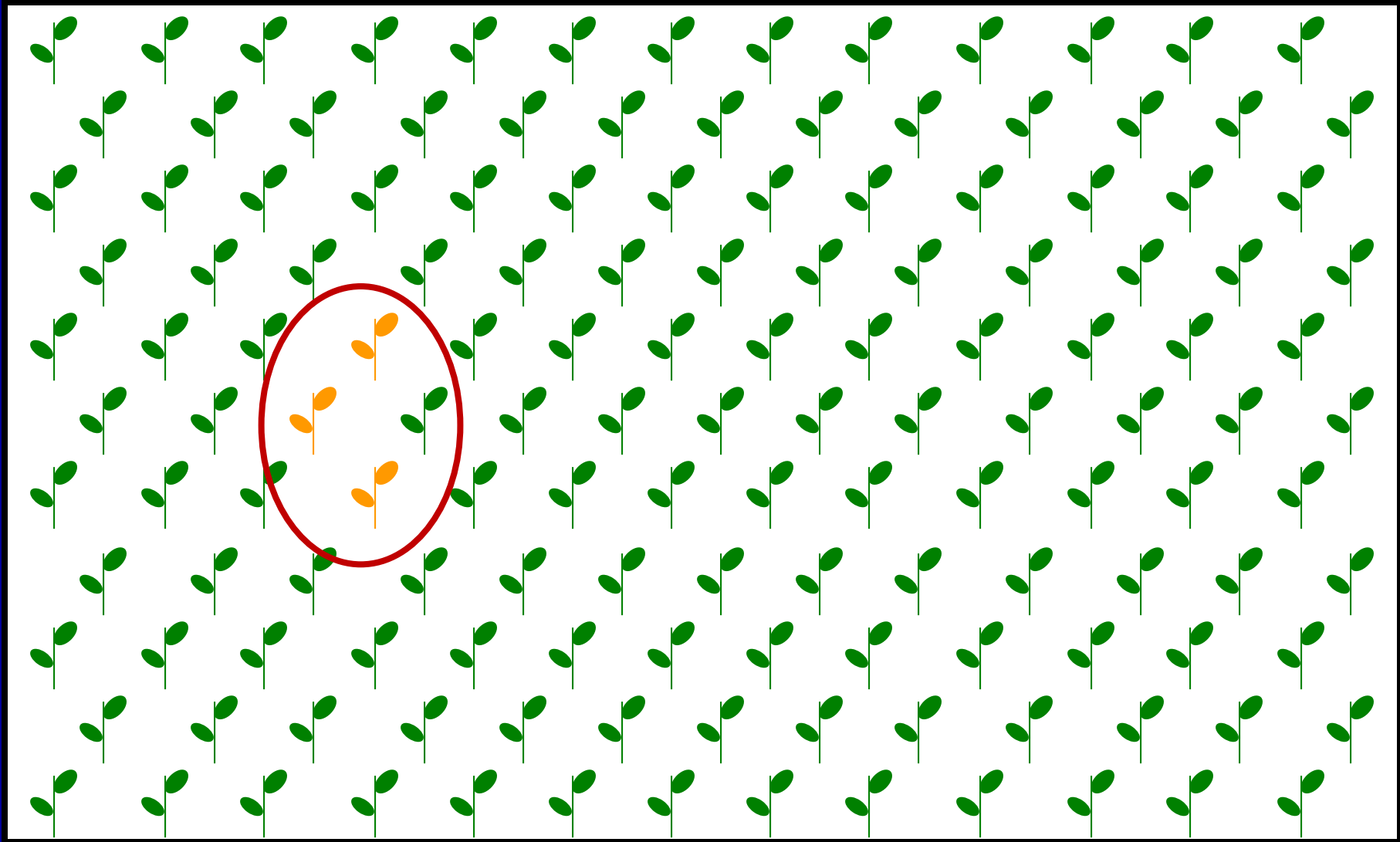
Selection Pressure

- Brings out herbicide resistance, but doesn't cause it
 - Natural genetic diversity in a weed population leads to an "alteration" in a plant that makes it resistant, instead of susceptible, to a herbicide
- Remember that repeated use of any control measure removes susceptible biotypes and leaves the resistant plants to reproduce
- Let's do a little selection pressure exercise...



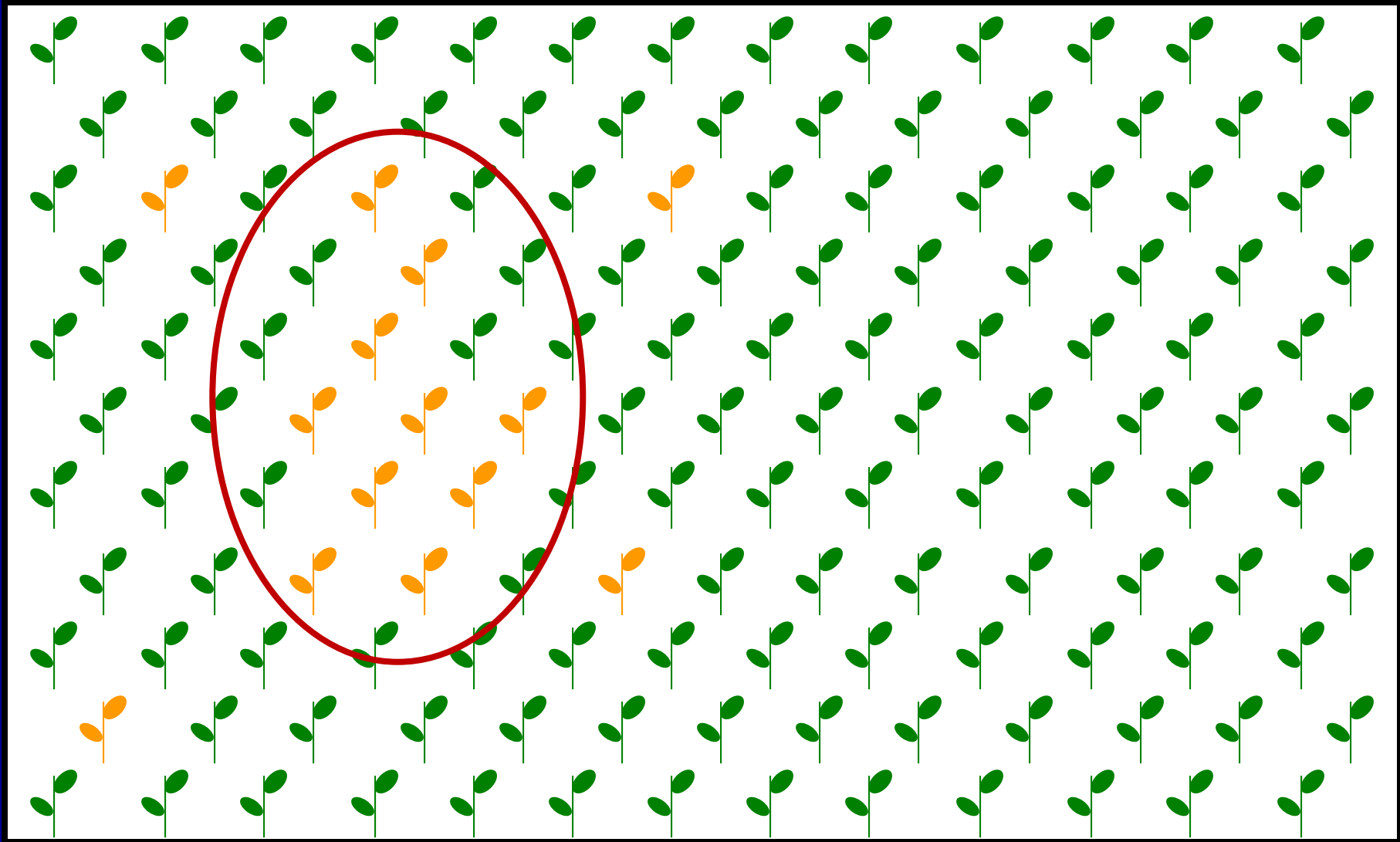
Application of herbicide "A"

Susceptible plants are killed
and resistant plant sets seed



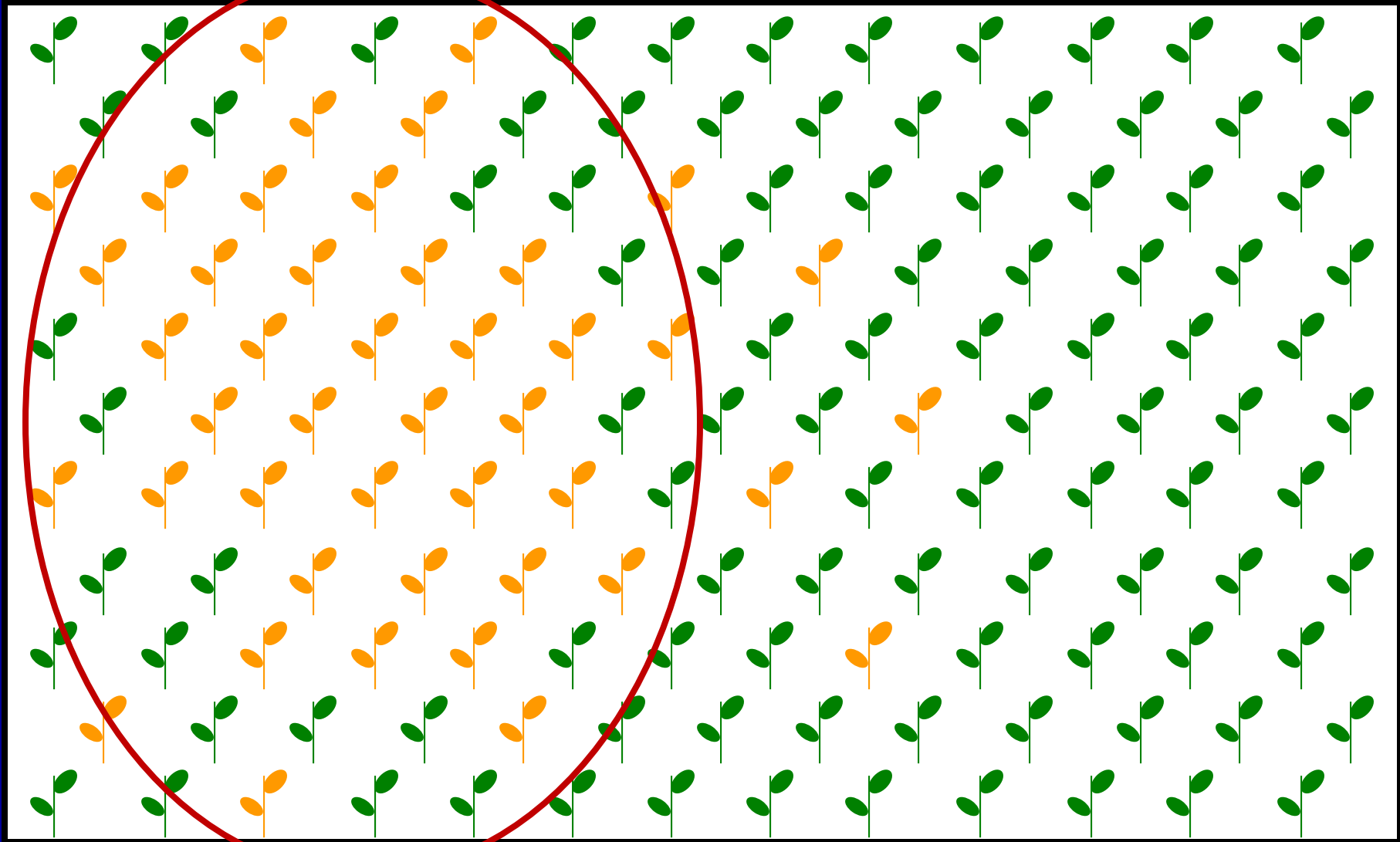
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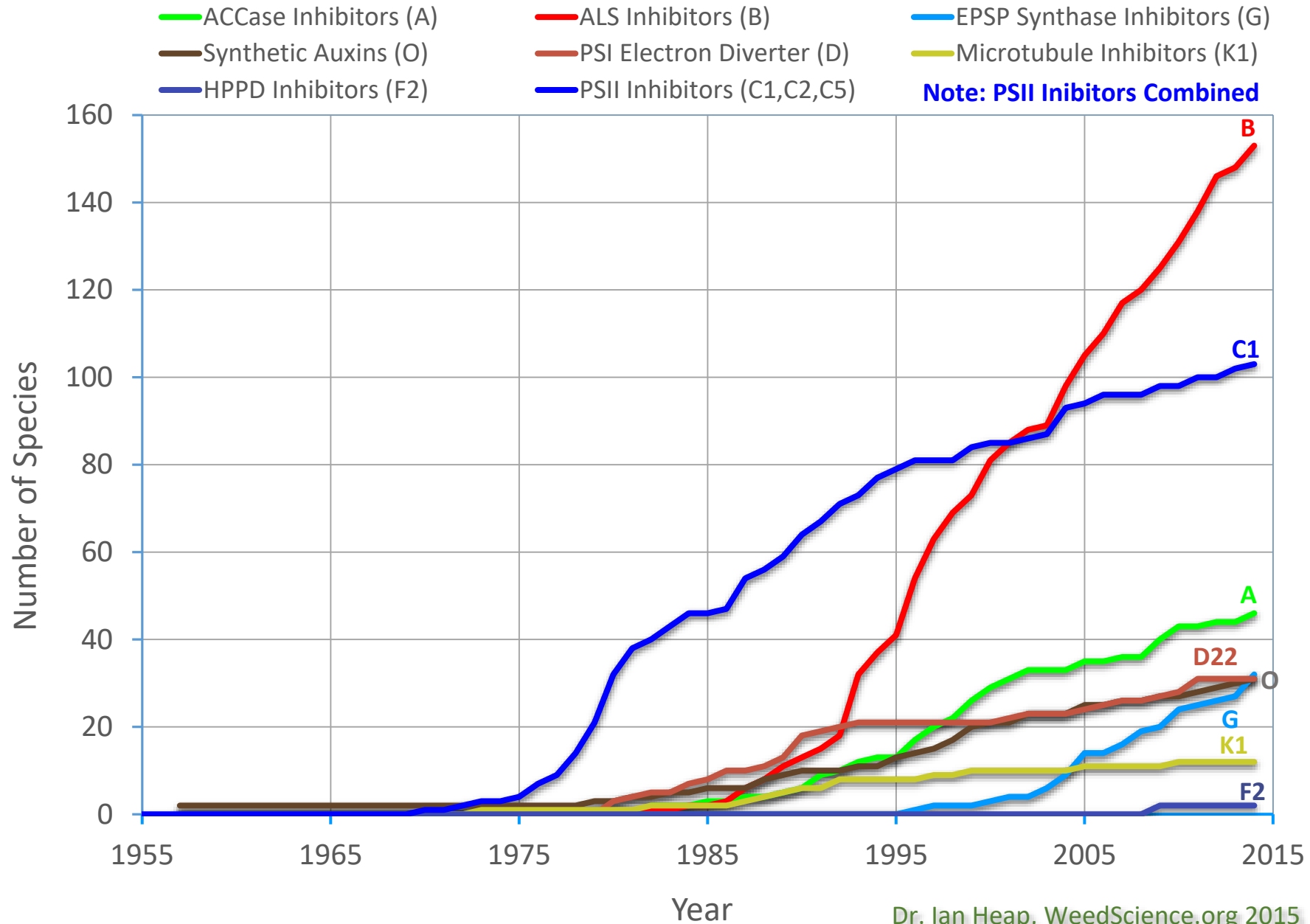
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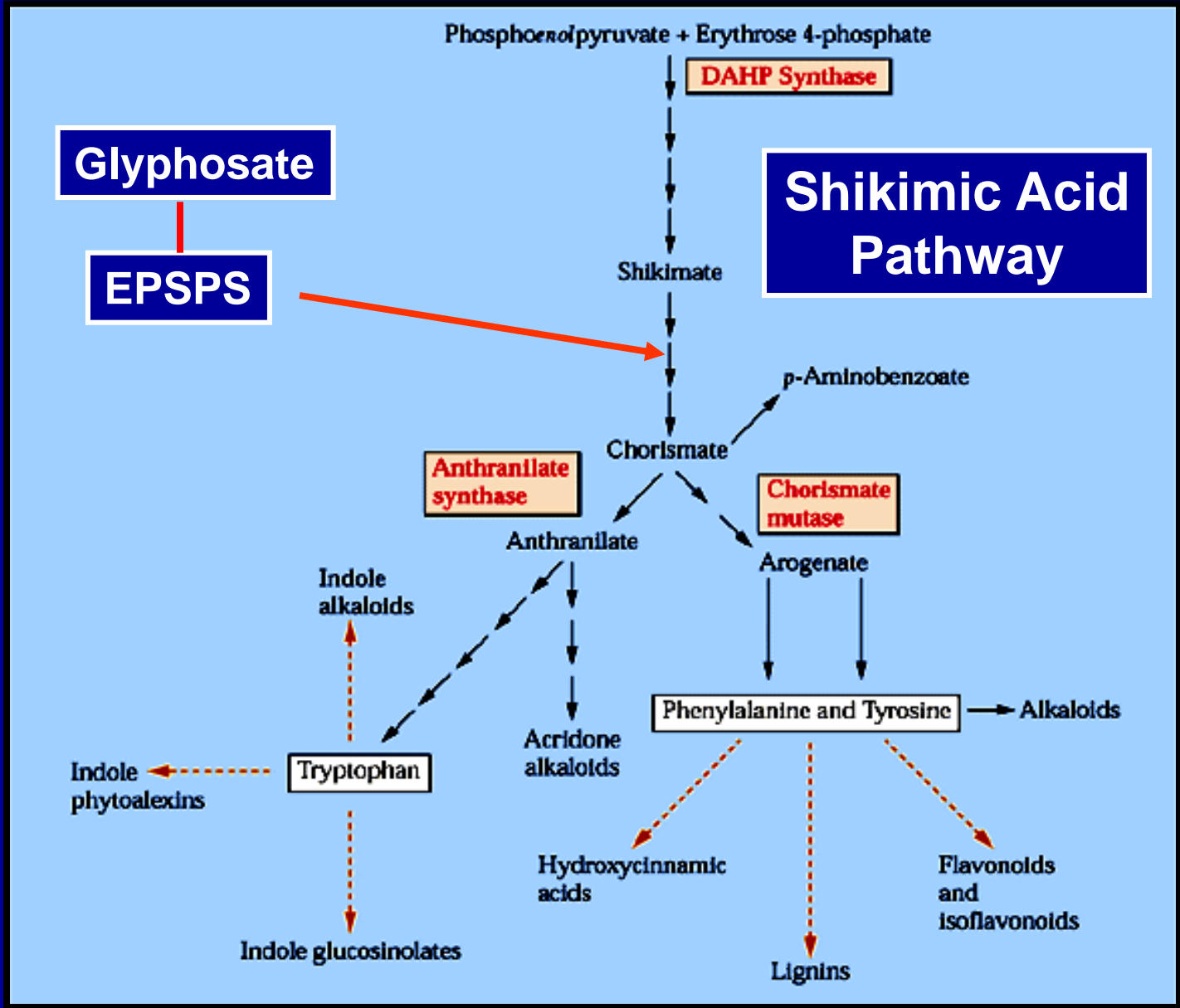


Herbicide Chemistry (MOA)

- Mode/mechanism of action:
 - The overall manner in which a herbicide affects a plant **at the tissue or cellular level**
 - Usually defined by a **specific enzymatic pathway** affected (can be broad or narrow)
 - eg. **photosynthetic inhibitors** (atrazine)
 - Mechanism of action usually references the **specific molecular site** in the plant
 - eg. inhibitors of **acetolactate synthase (ALS)** or **5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)**

Number of Herbicide Resistant Species Worldwide by MOA

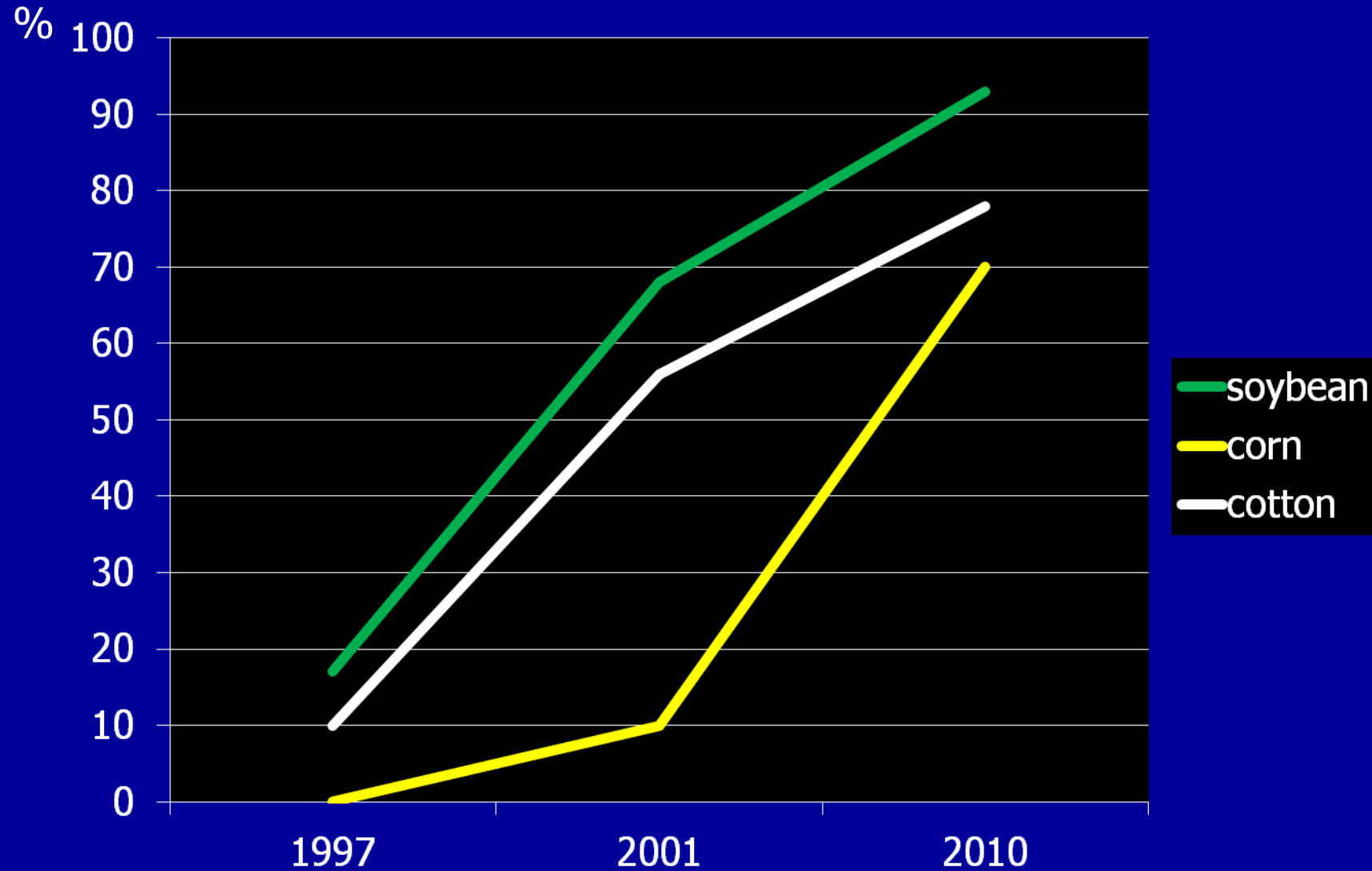




Glyphosate Resistant Crops

- Developed by **Monsanto scientists**
- Isolated a gene from a soil bacterium (***Agrobacterium strain CP4***) that produces an EPSPS enzyme with **a slightly different structure** than the EPSPS enzyme that commonly occurs in plants
 - This gene was **inserted into the DNA** of various crops
 - Glyphosate **does not bind** to this CP4 EPSPS enzyme, so it **continues to function** even when the herbicide is present in the cell
- The result: crops equipped with the **CP4 gene** are not killed **even when treated with glyphosate**

Percent of Soybean, Corn, and Cotton Acreage Planted with Glyphosate Resistant Crops in the United States



Glyphosate Resistant Crops

- A **second method** of producing glyphosate resistant crops involves **inserting a gene** that gives the plant the **ability to metabolize glyphosate**
- Commercial levels of **glyphosate resistance** have been achieved in **canola and tobacco** by using a gene isolated from another soil bacterium (***Pseudomonas strain LBr***) that allows the plant cell to produce the enzyme **glyphosate oxidoreductase**, resulting in a cell that **actively degrades any glyphosate** that translocates into the cell

Glyphosate Resistance

- So, in **glyphosate resistant crops**, resistance results from:
 - Insertion of the **bacterial gene** that provides for the type of **EPSPS** that **glyphosate cannot bind** to
 - Insertion of the **bacterial gene** that allows the plant to **metabolize glyphosate**

Glyphosate Resistance

- In **weeds**, glyphosate resistance may result from:
 - Mutation that causes **overexpression** or **amplification** of the **gene that produces EPSPS**, so each cell has an unusually large amount of **EPSPS enzyme**, making it **difficult to inhibit** the cell's ability to function normally

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 - Certain weeds may **absorb or translocate less glyphosate**, or do a better job of **sequestering** any glyphosate that is absorbed

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 - Certain weeds may **absorb or translocate less glyphosate**, or do a better job of **sequestering** any glyphosate that is absorbed
 - Out-crossing between **closely-related crops and weeds** may transfer the resistance gene to a surviving weed's progeny **via pollen flow**

Palmer Amaranth (a.k.a. Superweed!)



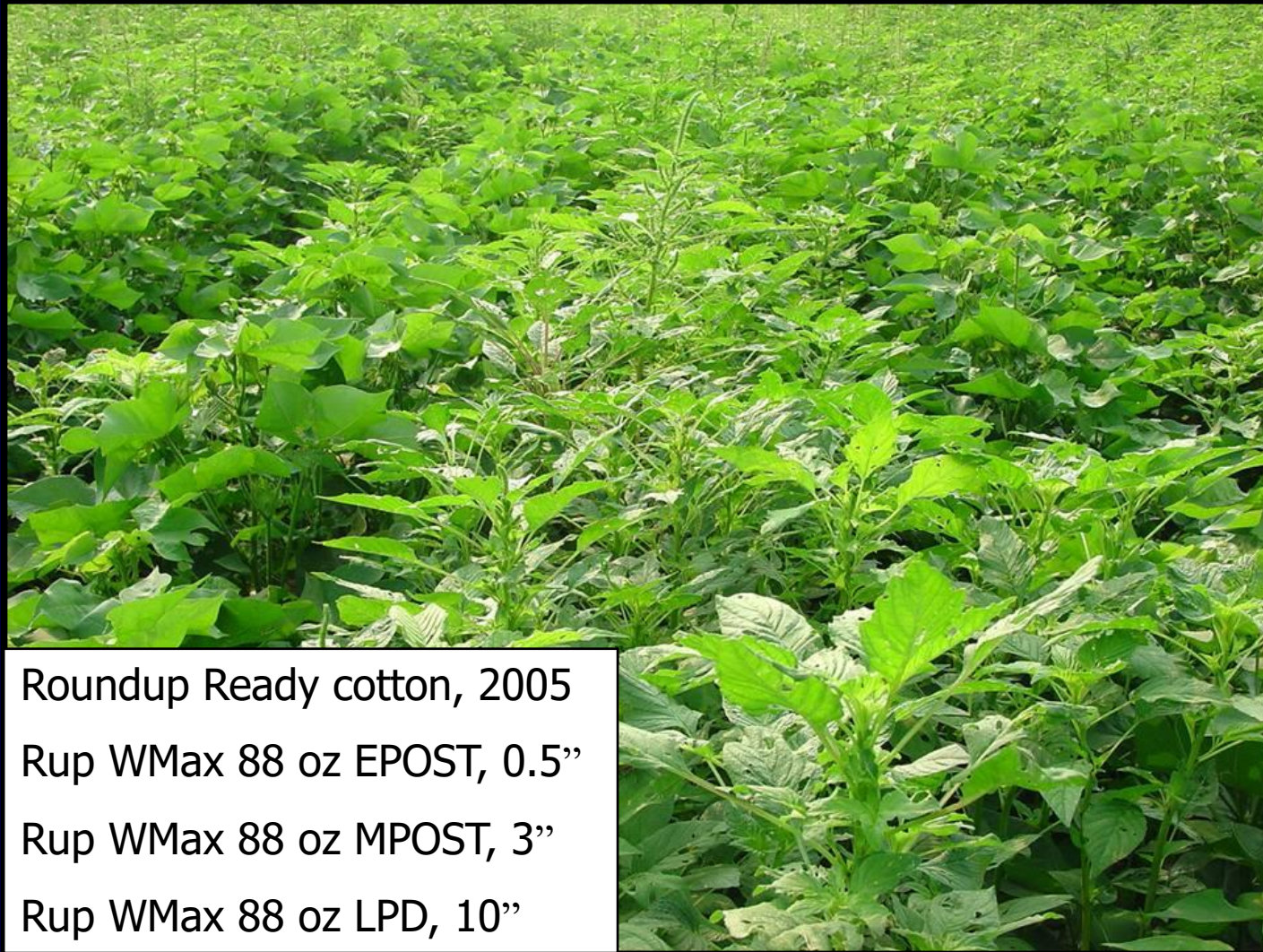
Cost = \$30-40/acre



Roundup Ready cotton, 2000
Rup WMax 22 oz EPOST, 0.5”



Cost = \$60-70/acre



Roundup Ready cotton, 2005

Rup WMax 88 oz EPOST, 0.5"

Rup WMax 88 oz MPOST, 3"

Rup WMax 88 oz LPD, 10"

Weeds that Naturally Tolerate Glyphosate in The West



Burning Nettle



Creeping
Buttercup



Panicle
Willowweed



Morningglory



Yellow
Nutsedge



Common
Lambsquarters



Purslane



Black Nightshade

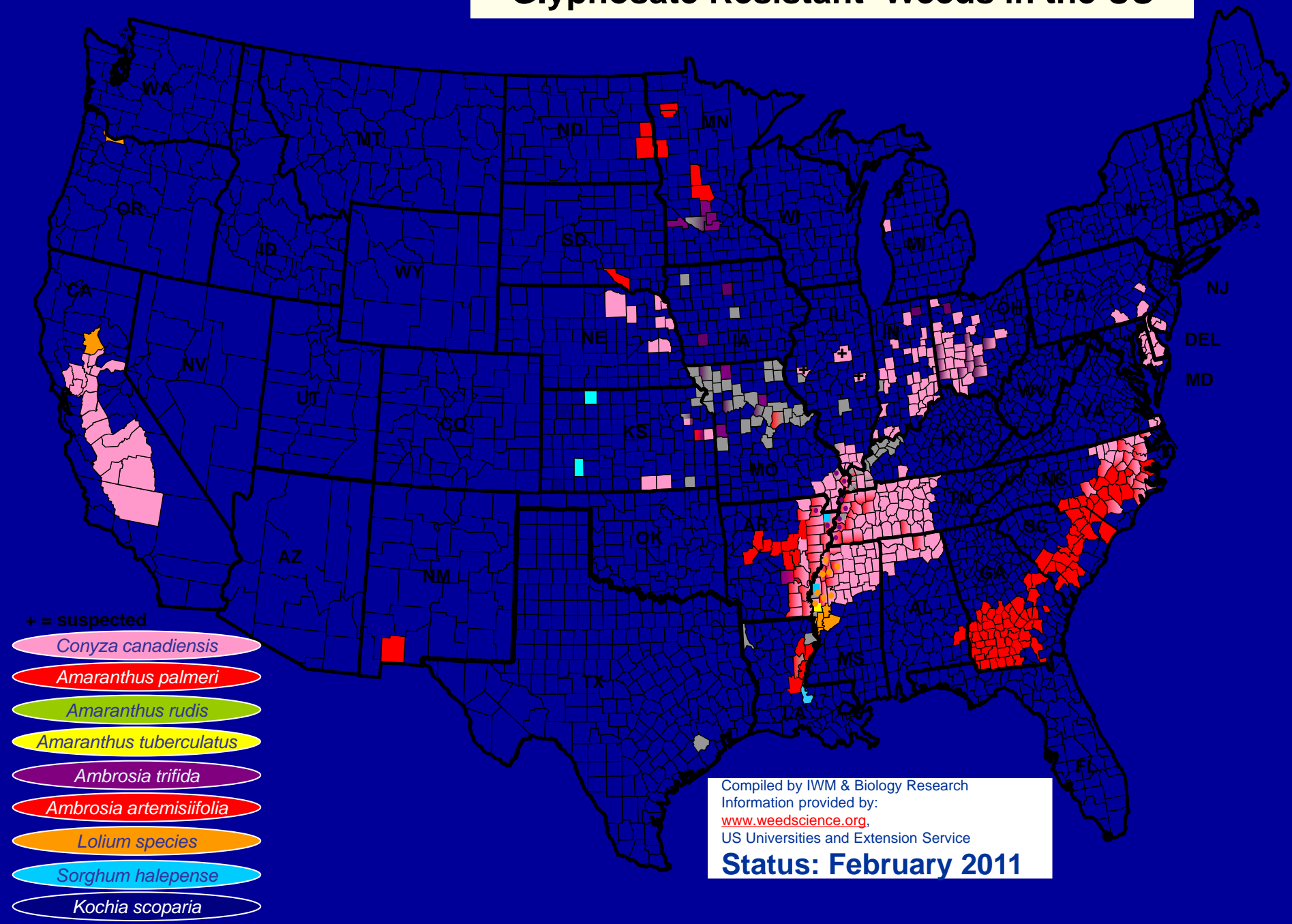


Redstem
Filaree



Common
Mallow

Glyphosate Resistant Weeds in the US



Compiled by IWM & Biology Research
Information provided by:
www.weedscience.org,
US Universities and Extension Service
Status: February 2011

Preventing and Managing Glyphosate Resistant Weeds

- Integrated Pest Management!
- Integrate glyphosate as **one of the tools** in a comprehensive weed control program
- Tank mix with or rotate with **another mode of action herbicide**
- Use **residual herbicide**
- **DO NOT** reduce rate



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