HERBICIDE TOLERANCE

Introduction

- Weeds are a constant problem in farmers fields.
- Weeds not only compete with crops for water, nutrients, sunlight, and space but also harbor insects and diseases; clog irrigation and drainage systems; undermine crop quality; and deposit weed seeds into crop harvests.
- If left uncontrolled, weeds can reduce crop yields significantly.
- Farmers can fight weeds with tillage, hand weeding, herbicides, or typically a combination of all techniques.
- Unfortunately, tillage leaves valuable topsoil exposed to wind and water erosion, a serious long-term consequence for the environment.
- For this reason, more and more farmers prefer reduced or no-till methods of farming.

Introduction

- Similarly, many have argued that the heavy use of herbicides has led to groundwater contaminations, the death of several wildlife species and has also been attributed to various human and animal illnesses.
- Researchers postulated that weed management could be simplified by spraying a single broad-spectrum herbicide over the field anytime during the growing season.

Herbicides

- Commonly known as Weed Killer.
- Chemical substances used to control or inhibit growth of unwanted plants (Weed).
- Selective herbicides control specific weed species, while leaving the desired crop relatively unharmed. (eg. 2,4-D, Pendimethalin, etc.)
- While Non-selective herbicides formulated to control both broadleaf and grass weeds. (eg. Paraquat, Glyphosate, etc.)

Development of Glyphosate and Glufosinate Herbicide Tolerant Plants

- Herbicide tolerant (HT) crops offer farmers a vital tool in fighting weeds and are compatible with no-till methods, which help preserve topsoil. They give farmers the flexibility to apply herbicides only when needed, to control total input of herbicides and to use herbicides with preferred environmental characteristics.

Technology Background

- ***How do these herbicides work?***
  These herbicides target key enzymes in the plant metabolic pathway, which disrupt plant food production and eventually kill it. So how do plants elicit tolerance to herbicides? Some may have acquired the trait through selection or mutation; or more recently, plants may be modified through genetic engineering.
- ***Why develop HT crops?***
  What is new is the ability to create a degree of tolerance to broad-spectrum herbicides - in particular glyphosate and glufosinate - which will control most other green plants.
- These two herbicides are useful for weed control and have minimal direct impact on animal life, and are not persistent. They are highly effective and among the safest of agrochemicals to use. Unfortunately, they are equally effective against crop plants. Thus, HT crops are developed to have a degree of tolerance to these herbicides.

How do Glyphosate and Glufosinate HT crops work?

- ***1. Glyphosate-tolerant crops***
  Glyphosate herbicide kills plants by blocking the EPSPS enzyme, an enzyme involved in the biosynthesis of aromatic amino acids, vitamins and many secondary plant metabolites.
- There are several ways by which crops can be modified to be glyphosate-tolerant.
- One strategy is to incorporate a soil bacterium gene that produces a glyphosate tolerant form of EPSPS. Another way is to incorporate a different soil bacterium gene that produces a glyphosate degrading enzyme.
- ***2. Glufosinate-tolerant crops***
  Glufosinate herbicides contain the active ingredient phosphinothricin, which kills plants by blocking the enzyme responsible for nitrogen metabolism and for detoxifying ammonia, a by-product of plant metabolism.
- Crops modified to tolerate glufosinate contain a bacterial gene that produces an enzyme that detoxifies phosphinothricin and prevents it from doing damage (Glutamine synthetase).

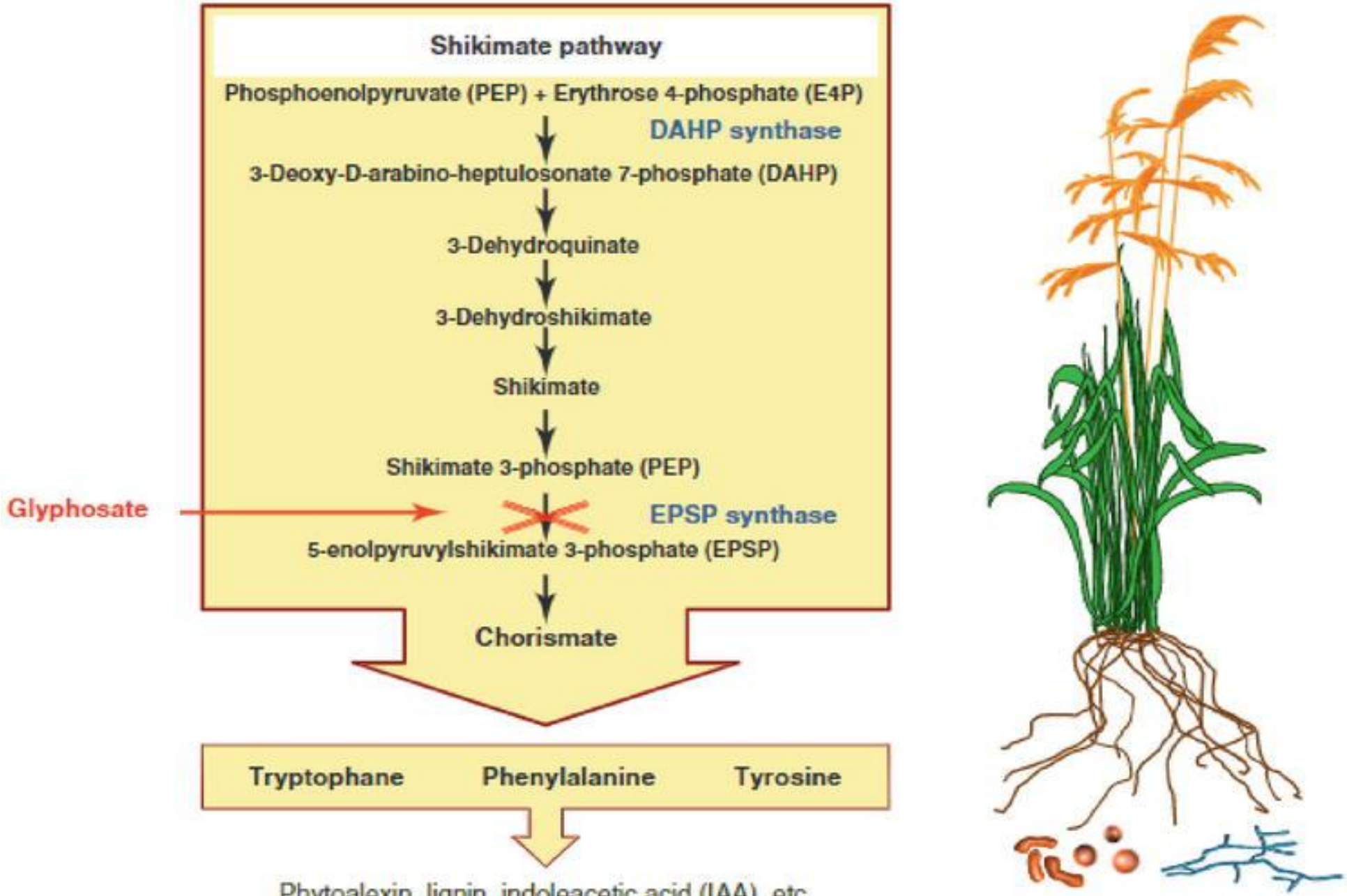
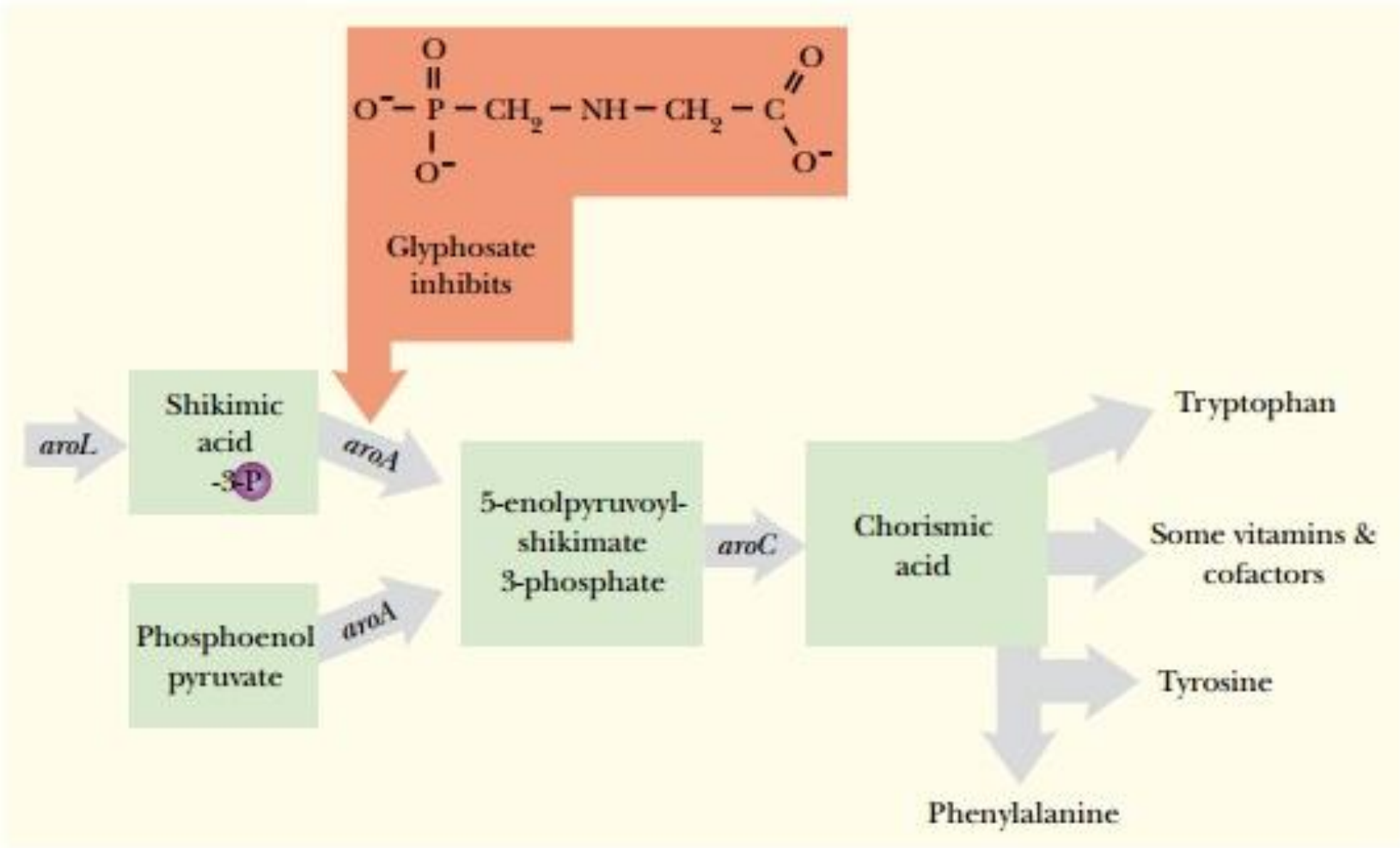

Shikimate pathway
Phosphoenolpyruvate (PEP) + Erythrose 4-phosphate (E4P)
DAHP synthase
3-Deoxy-D-arabino-heptulosonate 7-phosphate (DAHP)
3-Dehydroquinate
3-Dehydroshikimate
Shikimate
Shikimate 3-phosphate (PEP)
Glyphosate
EPSP synthase
5-enolpyruvylshikimate 3-phosphate (EPSP)
Chorismate
Tryptophane
Phenylalanine
Tyrosine
Phytoalexin, lignin, indoleacetic acid (IAA), etc.
Related plant and microbe responses: survival, growth, defense and secondary metabolite composition

FIGURE 14.12
Glyphosate Inhibits EPSPS in the Aromatic Pathway
The enzyme 5-enolpyruvoylshikimate-3-phosphate synthase (EPSPS) is the product of the *aroA* gene and makes 5-enolpyruvoylshikimate-3-phosphate, a precursor in the pathway to aromatic amino acids and cofactors. Glyphosate, an analog of phosphoenolpyruvate, inhibits EPSPS.



Classification by Mode of Action

1) Photosynthesis Inhibitors
2) Plant Growth Regulators
3) Amino Acid Biosysthesis Inhibitors
4) Growth Inhibitors
5) Pigment Inhibitors
6) Grass Specific herbicides
7) Membrane Disrupters

Selectivity by Differences in Crop and Weed Growth Stage

- Selectivity as a result of some morphological and physiological between crop and weed. Selectivity mainly for non-selective herbicides.

- When crop is older than the weed, crop will have an advantage on things such as:
  - thicker cuticle and leaf wax: less herbicide penetration.
  - more leaves: many angles - difficult to get a good coverage; insufficient translocation of herbicide.
  - higher growing point (meristem): growing point is protected, less likely for foliar herbicides to reach the growing point.
  - deeper root: less absorption: insufficient translocation.

E. Methods of application

- Refer to the ways herbicides can be applied:

1. Broadcast: applied over the entire field

2. Band: applied to a narrow strip over the crop row

3. Direct: applied between the rows of crop plants with little or no herbicide applied to the crop foliage

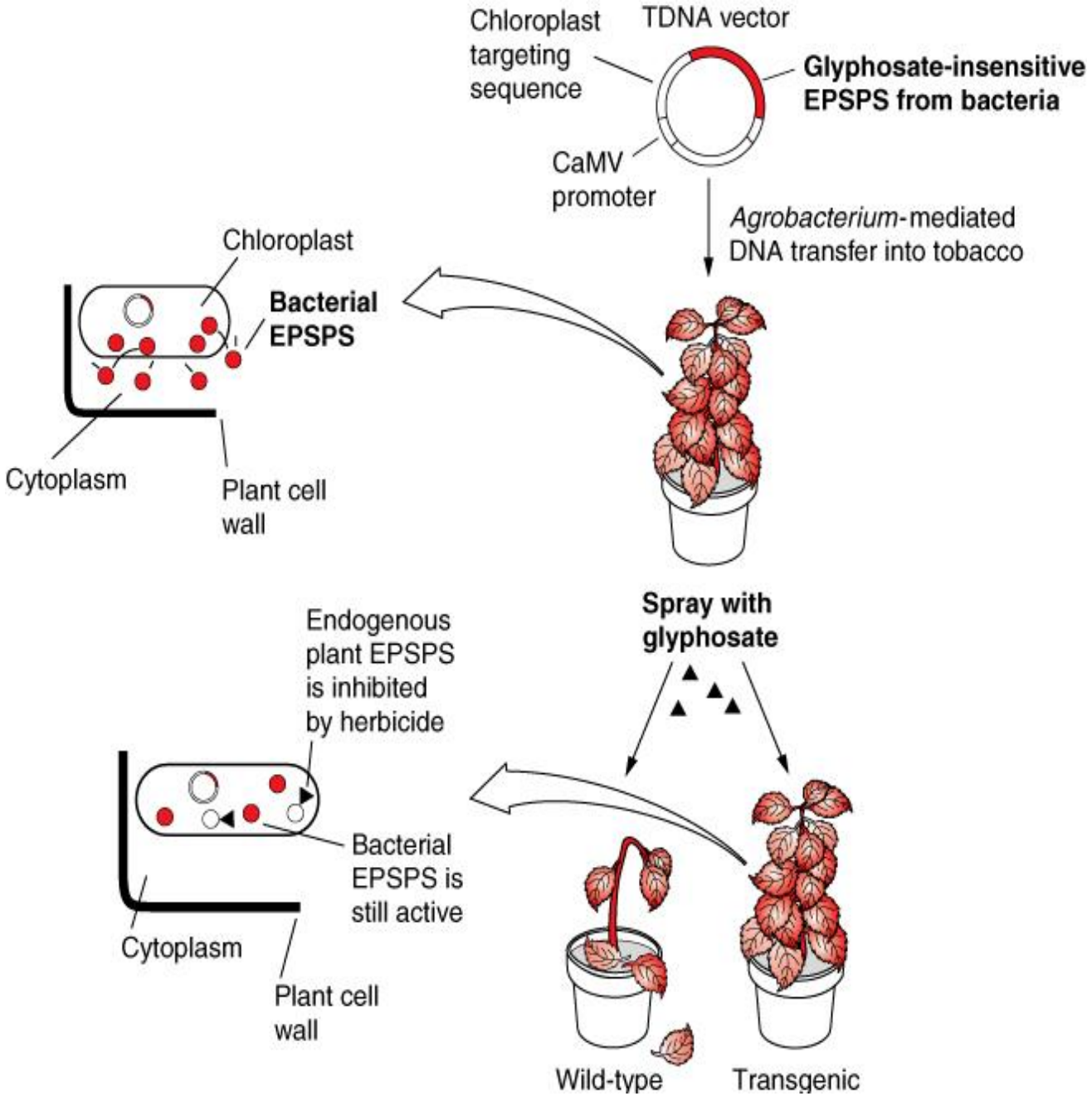
4. Spot treatment: applied to small, weed-infested areas within a field

An ideal herbicide is to posses the following characters : -

1. Capable of killing weeds with out affecting crop plants
2. Not toxic to animals & microorganisms
3. Rapidly trans located with in the target plant
4. Rapidly degraded in the soil

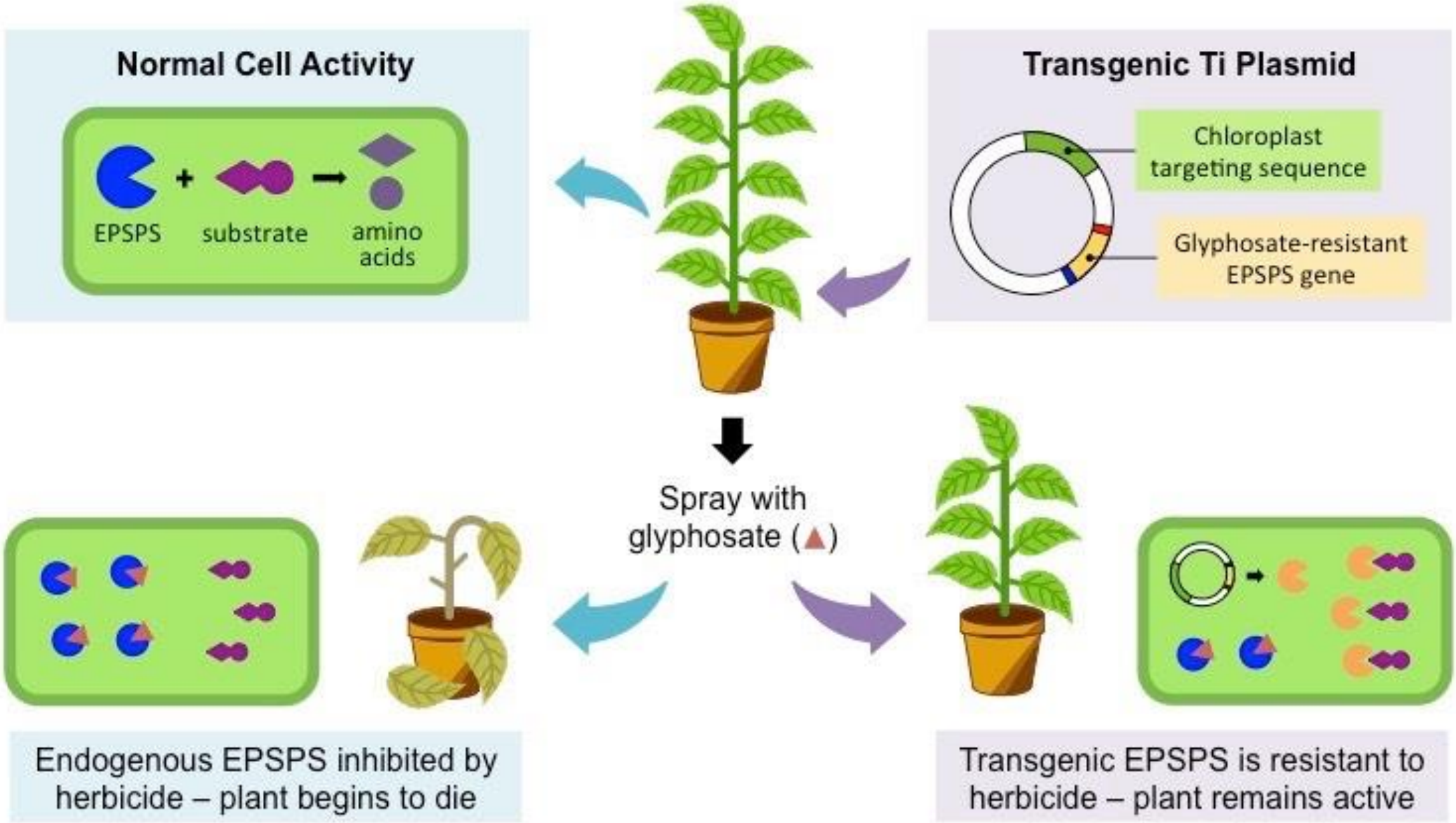
✓ Commercially available herbicides is that they can not discriminate weeds from the crop plants .

✓ For this reason , crops are also affected by herbicides hence the need to develop herbicide resistance plants



By using chemicals which target key enzymes in the plant metabolic pathway, like Glyphosate-tolerant crops (which block the EPSPS enzyme involved in the biosynthesis of amino acids)

and Glufosinate-tolerant crops (which contain Phosphinothricin which blocks the enzyme responsible for nitrogen metabolism and detoxifying ammonia), and the traditional crop rotation technique, we can get rid of those pesky weeds


Normal Cell Activity
EPSPS
substrate
amino acids
Transgenic Ti Plasmid
Chloroplast targeting sequence
Glyphosate-resistant EPSPS gene
Spray with glyphosate (▲)
Endogenous EPSPS inhibited by herbicide – plant begins to die
Transgenic EPSPS is resistant to herbicide – plant remains active

How do Glyphosate and Glufosinate HT crops work?

- Other methods by which crops are genetically modified to survive exposure to herbicides including:

1) producing a new protein that detoxifies the herbicide;

2) modifying the herbicide's target protein so that it will not be affected by the herbicide; or

3) producing physical or physiological barriers preventing the entry of the herbicide into the plant. The first two approaches are the most common ways scientists develop herbicide tolerant crops.

In plants, **glyphosate** disrupts the shikimic acid **pathway** through inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase. The resulting deficiency in EPSP production leads to reductions in aromatic amino acids that are vital for protein synthesis and plant growth.

Round up

- Roundup is **a popular weedkiller manufactured by Monsanto**, now owned by Bayer. Its active ingredient is glyphosate — an ingredient that is becoming increasingly controversial around the globe. ... Farmers widely use glyphosate-based weedkillers, including Roundup, to protect their crops from destructive weeds.
- Introduced Glyphosate resistant soybean in 1996 and corn in 1998.
- **Bayer will stop selling Roundup for residential use in 2023**, an effort to prevent future cancer lawsuits. The company has lost several significant lawsuits from plaintiffs who alleged glyphosate gave them cancer. The new decision is an attempt to minimize future liability

Roundup Ready Soybeans (RR soybeans) are **genetically engineered soybeans** that have had their DNA altered to allow them to withstand the herbicide glyphosate (the active ingredient in Monsanto's herbicide Roundup). They are also known as "glyphosate tolerant" soybeans

Roundup Ready

- Roundup Ready soybean was the first crop plant produced by Monsanto.
- Today, 90% of the soybean crop in the USA consists of Roundup Ready® plants.
- You can't buy soybean products that don't come from genetically modified plants.

Roundup Ready Crops

- In 1996, genetically modified *Roundup Ready* soybeans resistant to Roundup became commercially available, followed by *Roundup Ready* corn in 1998
- Current *Roundup Ready* crops include soy, maize (corn), canola, sugar beet, and cotton, with wheat and alfalfa still under development.

Roundup Ready® CORN 2

Safety aspects of herbicide tolerant technology

- ***Toxicity and Allergenicity***
  Government regulatory agencies in several countries have ruled that crops possessing herbicide tolerant conferring proteins do not pose any other environmental and health risks as compared to their non-GM counterparts.
- Introduced proteins are assessed for potential toxic and allergenic activity in accordance with guidelines developed by relevant international organizations.
- They are from sources with no history of allergenicity or toxicity; they do not resemble known toxins or allergens; and they have functions, which are well understood.
- ***Effects on the Plants***
  The expression of these proteins does not damage the plant's growth nor result in poorer agronomic performance compared to parental crops. Except for expression of an additional enzyme for herbicide tolerance or the alteration of an already existing enzyme, no other metabolic changes occur in the plant.

Safety aspects of herbicide tolerant technology

- ***Persistence or invasiveness of crops***
  A major environmental concern associated with herbicide tolerant crops is their potential to create new weeds through outcrossing with wild relatives or simply by persisting in the wild themselves.
- This potential, however, is assessed prior to introduction and is also monitored after the crop is planted.
- The current scientific evidence indicates that, in the absence of herbicide applications, GM herbicide-tolerant crops are no more likely to be invasive in agricultural fields or in natural habitats than their non-GM counterparts
- The herbicide tolerant crops currently in the market show little evidence of enhanced persistence or invasiveness

Advantages of Herbicide Tolerant Crops

- Excellent weed control and hence higher crop yields;
- Flexibility – possible to control weeds later in the plant's growth;
- Reduced numbers of sprays in a season;
- Reduced fuel use (because of less spraying);
- Reduced soil compaction (because of less need to go on the land to spray);
- Use of low toxicity compounds which do not remain active in the soil; and
- The ability to use no-till or conservation-till systems, with consequent benefits to soil structure and organisms

Disadvantages of Herbicide-Tolerant Crops

- **Excessive Herbicide Use -**Because there is no risk of killing the crops with the herbicides, use of herbicides carries environmental risks, possibly leading to increased pollution. Possible effects include contamination of groundwater, carrying risks for human health.
- **New Weeds**
- **Biodiversity Impact -** In particular, there are concerns of affecting the diversity of the plant species that are present on farmland where herbicide-resistant crops are planted.
- **Gene Flow -** The use of herbicide-resistant crops could potentially affect other crops on the farm that are not herbicide-resistant, leading to a danger of transferring genes to a crop that a farmer wants to keep non-herbicide-resistant,

Current status of herbicide tolerance

- From 1996 to 2018, HT crops consistently occupied the largest planting area of biotech crops.
- In 2018 alone, HT crops occupied 87.5 million hectares or 45% of the 191.7 million hectares of biotech crops planted globally.
- The most common are the glyphosate and glufosinate tolerant varieties. Some countries have approved major HT (with single and stacked genes) crops for food, feed, and/or cultivation.
- For the first 21 years of commercialization (1996-2016), benefits from herbicide tolerant crops are valued at US$ 89.02 billion, 47.8% of global biotech crop value of US$ 186.1 billion, and for 2016 alone at US$ 8.44 billion or 46.4% of global value of US$ 18.2 billion.
- Another class of very potent and broad spectrum herbicide is the Sulfonylurea inhibiting the enzyme **Acetolactase synthase** (ALS), the first enzyme in the pathway for biosynthesis of the EAA (Isoleucine, leucine and valine in plants).

Most common GE traits

Most GE crops on the market were designed to either be resistant to herbicides, insect pests or both.

PEST RESISTANCE

A gene from the microbe Bacillus thuringiensis (Bt) is inserted in cotton, corn and other crops, allowing the plants to produce an insecticide that kills caterpillar pests. Bt in spray form is widely used in organic farming.

HERBICIDE RESISTANCE

Corn, soy, canola, cotton and other crops have been genetically engineered with a bacterial gene to be immune to the weed-killer glyphosate, sold as Roundup. When farmers spray their fields with Roundup, the weeds are killed but the crops survive.

Protocol for Glyphosate tolerant Potato Plant

Introduction of 'bar' gene through vector

↓

Cell culture of potato with 'bar' gene

↓

Herbicide tolerant potato cells

↓

In vitro culture

↓

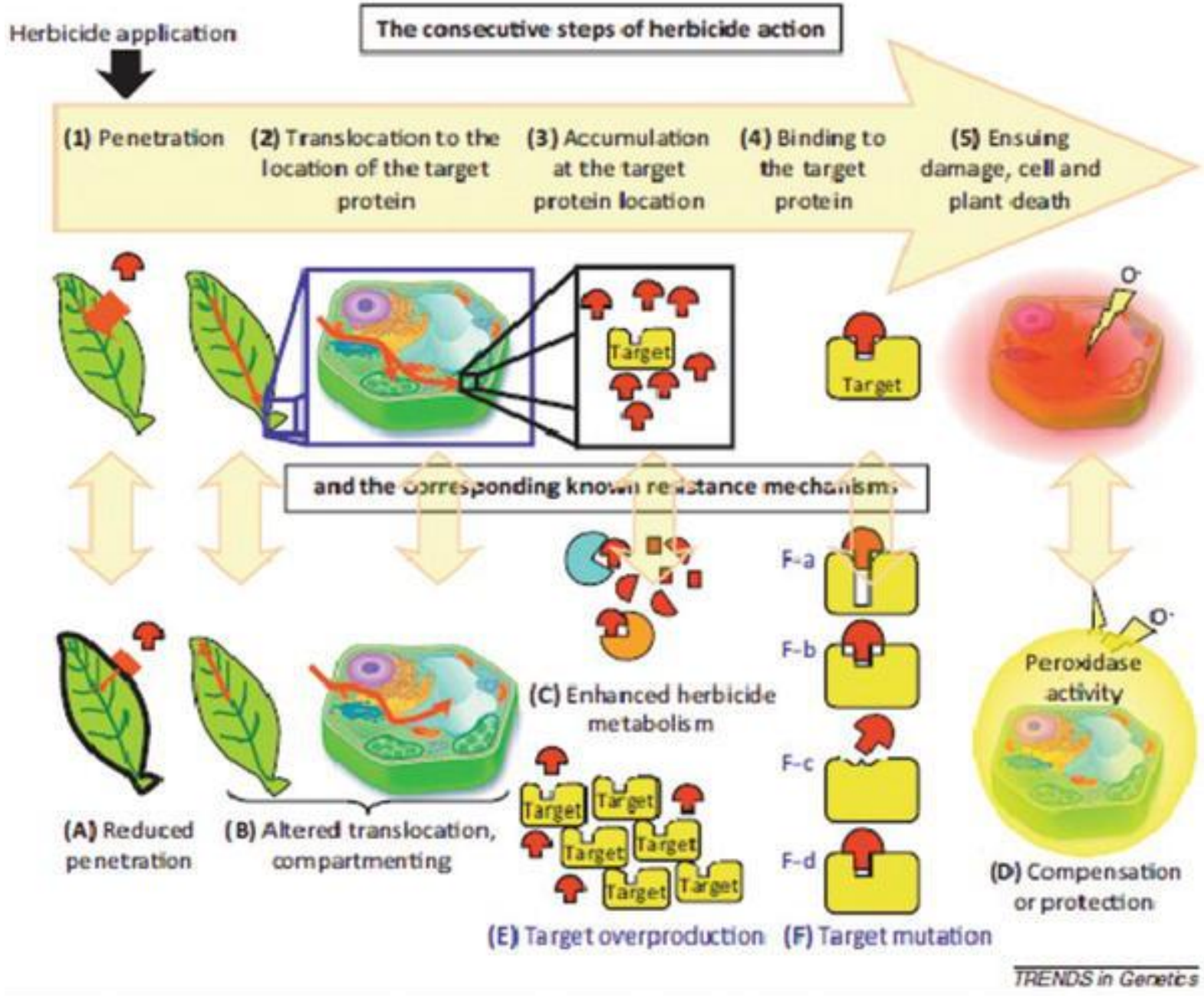
Callus → Organogenesis

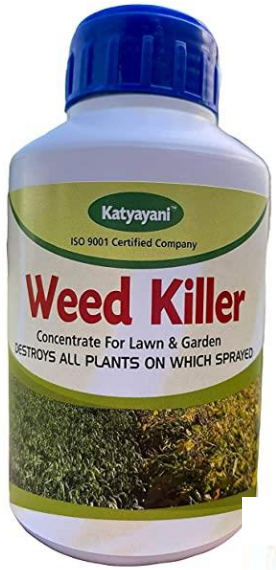
↓

Development of Herbicide tolerant transgenic plants

Figure 4.24: Glyphosate Tolerant Potato Plant

A gene which **confers resistance to the herbicide bialaphos** (bar) has been characterized. The bar gene was originally cloned from Streptomyces hygroscopicus, an organism which produces the tripeptide bialaphos as a secondary metabolite.


Herbicide application
The consecutive steps of herbicide action
(1) Penetration
(2) Translocation to the location of the target protein
(3) Accumulation at the target protein location
(4) Binding to the target protein
(5) Ensuing damage, cell and plant death
Target
O·
and the corresponding known resistance mechanisms
F-a
F-b
F-c
F-d
O·
Peroxidase activity
(A) Reduced penetration
(B) Altered translocation, compartmenting
(C) Enhanced herbicide metabolism
(D) Compensation or protection
(E) Target overproduction
(F) Target mutation
TRENDS in Genetics


Katyayani
ISO 9001 Certified Company
Weed Killer
Concentrate For Lawn & Garden
DESTROYS ALL PLANTS ON WHICH SPRAYED


BAYER
ADVANCED
All-in-One
Weed Killer
for Lawns
Kills Lawn Weeds
plus Crabgrass
BATTERY
POWERED
SPRAYER


GALLON
CONCENTRATE
ORTHO
WEED B GON
WEED KILLER
NORTHERN &
SOUTHERN LAWNS
KILLS
250+
WEEDS
NEW GREEN
GOBBLER
20% VINEGAR
WEED KILLER
Kills Weeds
And Grass
Spectracide
WEED
& GRASS
KILLER
CONCENTRATE