

Pest and Herbicide Resistance in Plants



Pest resistance



Pests and Crops

- *Insects attack agricultural products in field and during storage*
- *Even with the use of pesticides 13% of the harvested plant products are lost to insect pests*
- *Insects belonging to **Coleoptera**, **Lepidoptera** and **Diptera** are major pests*
- *Broad spectrum pesticides are used but have harmful side effects*
- *Problem tackled with the help of Genetic Engineering*



Orders of Insects



Lepidoptera



Diptera



Coleoptera



Pest Resistance

- *Genetic transformation has led to enhanced resistance to insects and pathogens*
- *Rapidly getting commercialized*
- *Chemical free and economically viable approach*
- *Overall process of genetic transformation involves introduction, integration and expression of foreign gene in the recipient host plant.*



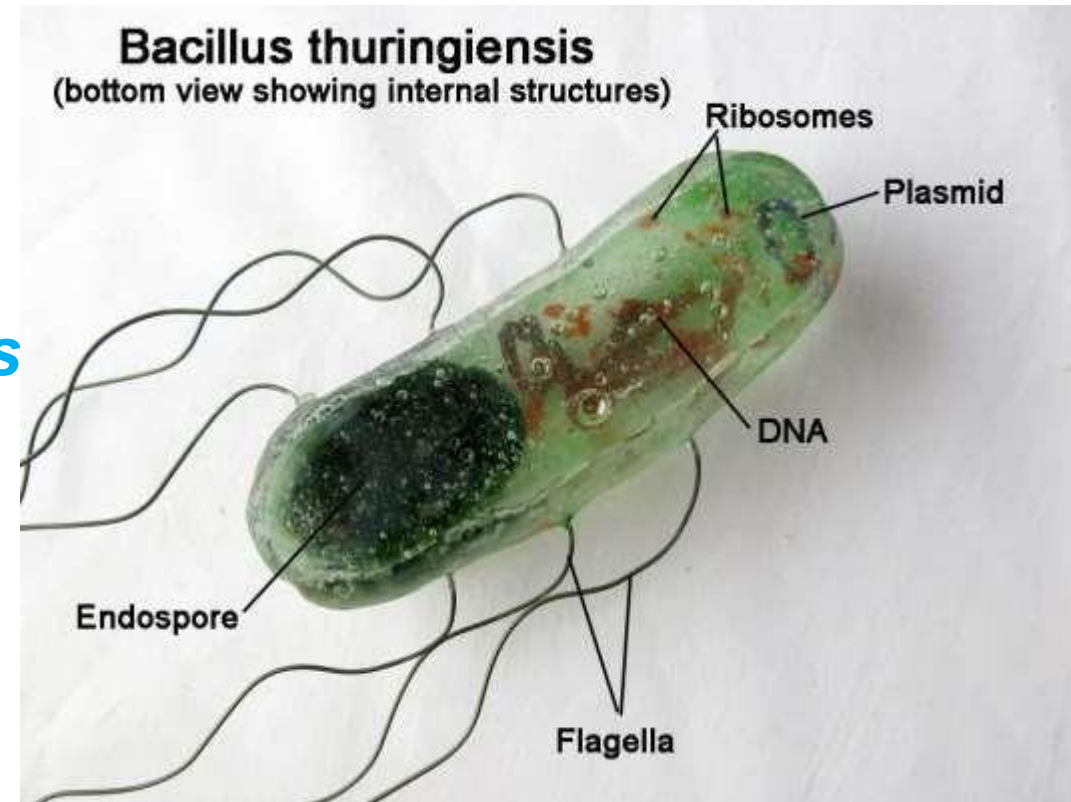
Pest Resistance



- *First reported in tobacco and tomato.*
- *Today, insect resistance transgenes can be introduced to plants.*
- *Approx 40 different genes conferring insect resistance have been obtained from microorganisms.*
- *Example: **Bt** gene from **Bacillus Thuringiensis** and **Ipt** gene from **Agrobacterium tumefaciens***

Bacillus thuringiensis (Bt gene)

- *Free-living, gram-positive soil bacterium*
- *Employed as an insecticide from over 20 years*
- *Specificity towards lepidopteran pests*
- *Environmentally safe*





Bacillus thuringiensis (Bt gene)

- *Bacillus thuringiensis* discovered by **Ishiwatari** in 1901
- Bacterium produces insecticidal crystal protein (ICP) also known as **Cry protein**
- They are a class of endotoxin – **δ endotoxins.**
- Upon sporulation, *B. thuringiensis* forms crystals of proteinaceous insecticidal δ-endotoxins which are encoded by cry genes
- In most strains of *B. thuringiensis* the cry genes are located on the plasmid

Bt Spray

- Earlier, Bt sprays were used to control insect attacks on plants
- Problems faced were:
- High-cost of production of insecticide
- Instability of the protoxin crystal proteins in field conditions
- Multiple applications were required
- *Bt* products such as sprays are rapidly washed away by rain, and degrade under the sun's UV rays.
- To tackle this, Bt gene has been introduced into plants



MONSANTO

BIO TECHNOLOGY

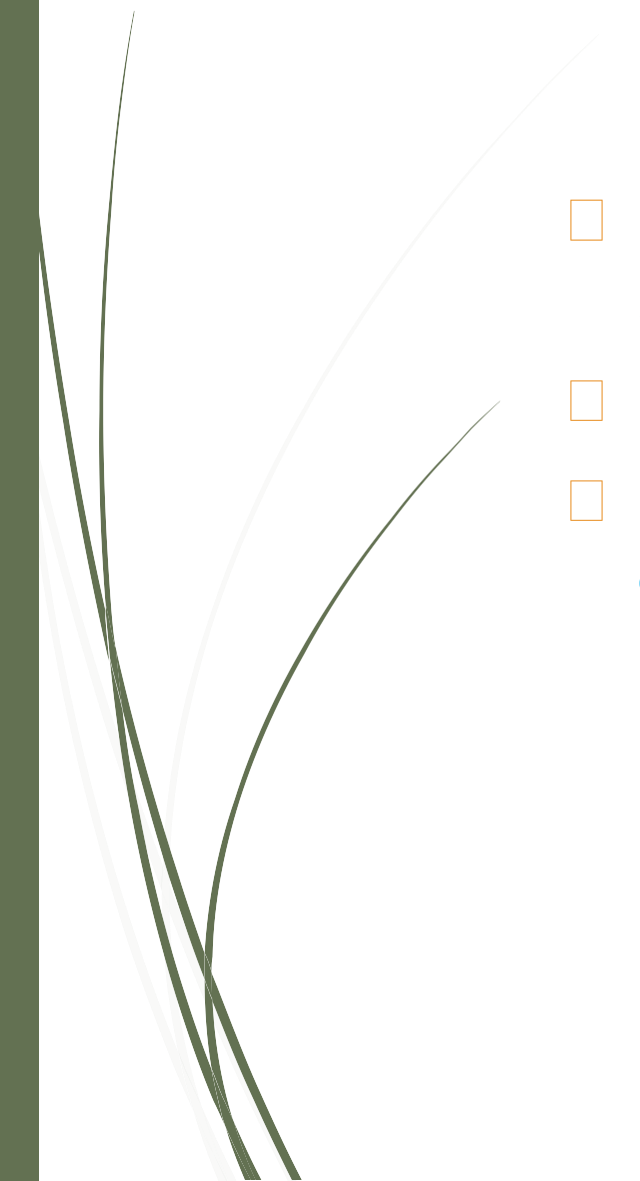
innovation – collaboration – speed



- *The Monsanto Company is a U.S.-based multinational agricultural biotechnology corporation*
- *It is the world's leading producer of the herbicide glyphosate, marketed as "Roundup"*
- *Monsanto is also the leading producer of genetically engineered(GE)seed; it sells 90% of the US's GE seeds*
- *The Company is focused on enabling both small-holder and large-scale farmers to produce more food, feed, fibre from their land*



History

- *First group to report success was Belgian Biotech Company, Plant Genetic Systems*
 - *Truncated Bt gene inserted into tobacco plants*
 - *Produced enough endotoxin to kill Manduca sexta larvae eating its leaf*
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Bt Cotton

- The gene coding for Bt toxin has been inserted into cotton as a transgene
- the main pests in commercial cotton are lepidopteran larvae
- eliminates the need to use large amounts of broad-spectrum insecticides to kill lepidopteran pests
- Bt cotton is ineffective against many cotton pests such as plant bugs, stink bugs, and aphids





Mechanism of Action

- Sporulating bt produces non-toxic proteinaceous endotoxin called protoxin
- Upon hydrolysis under alkaline pH of the insect gut, produces insecticidally active peptides
- The active peptides attack specifically brush border membrane of gut epithelial cells
- Paralyzes the insect and eventually kills it
- Different strains produce different endotoxins specific for different insects
- Bt toxin is safe to humans and other mammals



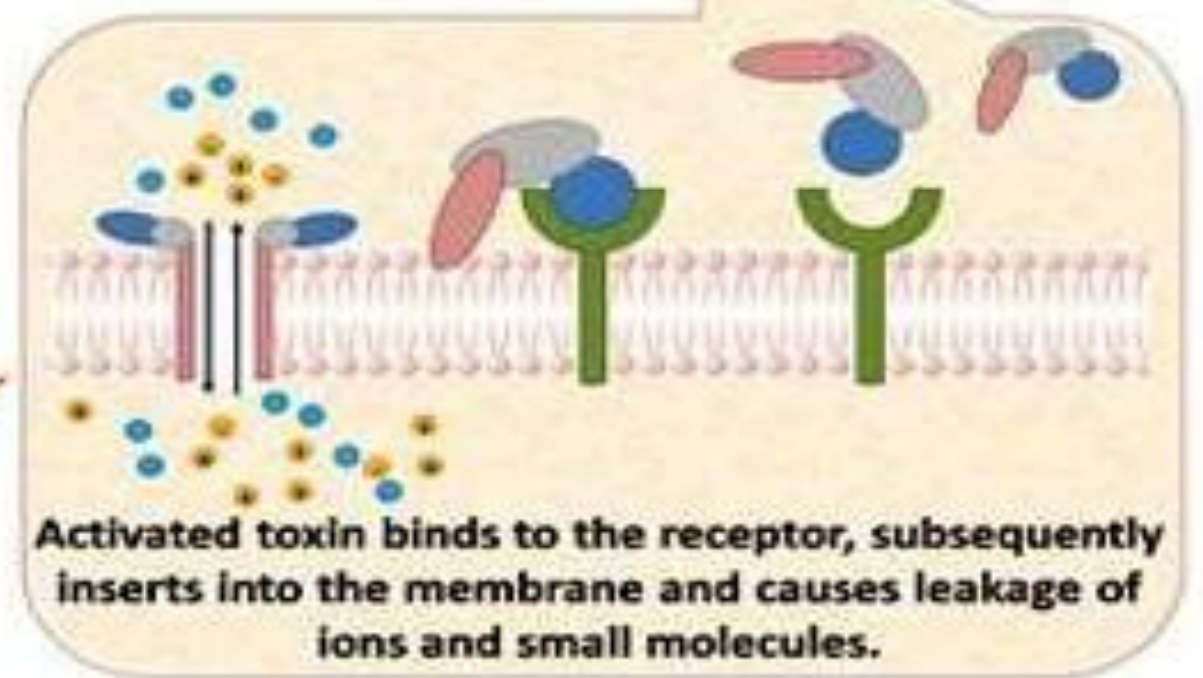
Crystals and spores are ingested by insect larvae.



Toxins are activated to active form by gut enzymes.




Midgut membrane damage leads to starvation or septicemia.



Activated toxin binds to the receptor, subsequently inserts into the membrane and causes leakage of ions and small molecules.




Mechanism of Action

- Bt cotton was created through the addition of genes encoding toxin crystals in the Cry group of endotoxin
 - When insects attack and eat the cotton plant the Cry toxins are dissolved due to the high pH level of the insects stomach
 - The dissolved and activated Cry molecules bond to cadherin-like proteins on cells comprising the brush border molecules
 - The epithelium of the brush border membranes separates the body cavity from the gut whilst allowing access for nutrients
- 



Mechanism of Action

- The Cry toxin molecules attach themselves to specific locations on the cadherin-like proteins present on the epithelial cells of the midge
 - ion channels are formed which allow the flow of potassium
 - Regulation of potassium concentration is essential and, if left unchecked, causes death of cells
 - Due to the formation of Cry ion channels sufficient regulation of potassium ions is lost and results in the death of epithelial cells
 - The death of such cells creates gaps in the brush border membrane.
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Problems of Bt cotton cultivation

- Seeds are not reusable**
- Development of resistance to Bt toxins in pests**
- availability of fake seeds of Bt cotton in the market**
- gene contamination**

Herbicide Resistance



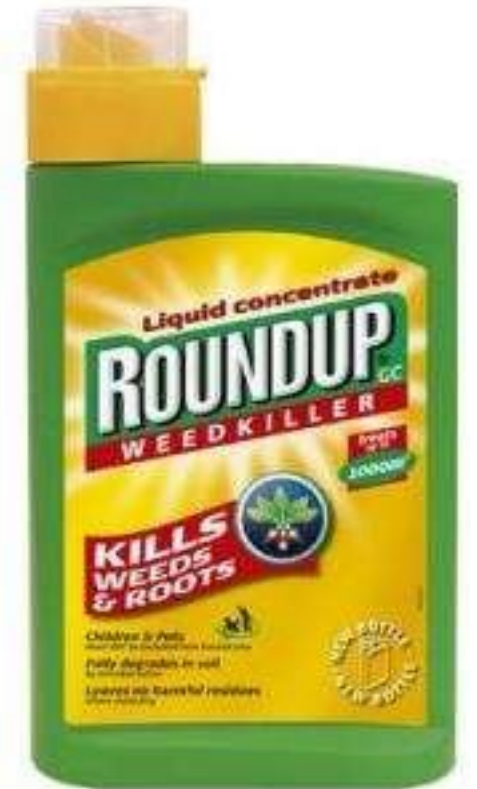


Weeds and Crops

- Weeds compete with crop plants for nutrient, light, moisture
- Cause considerable decline in quality and yield
- Herbicidal compounds are available for weed management
- Selection of herbicide depends on:
 - I. Its effectiveness against the weeds
 - II. Crop sensitivity
 - III. Herbicide treatment

Problem with herbicides

- Several classes of herbicides are effective for broad spectrum weed control
- Act by inactivating vital enzymes (involved in photosynthesis)
- Hence non-selective and kill or damage crop plants
- To face this problem, herbicide resistant plants are generated





Herbicide resistant plants

□ Three approaches:

- A. Modification of plant enzyme target of herbicidal action to render it insensitive to herbicide*
- B. Overproduction of the unmodified target protein permitting normal metabolism to occur even in herbicide presence*
- C. Introduction of an enzyme to degrade herbicide prior to its action*

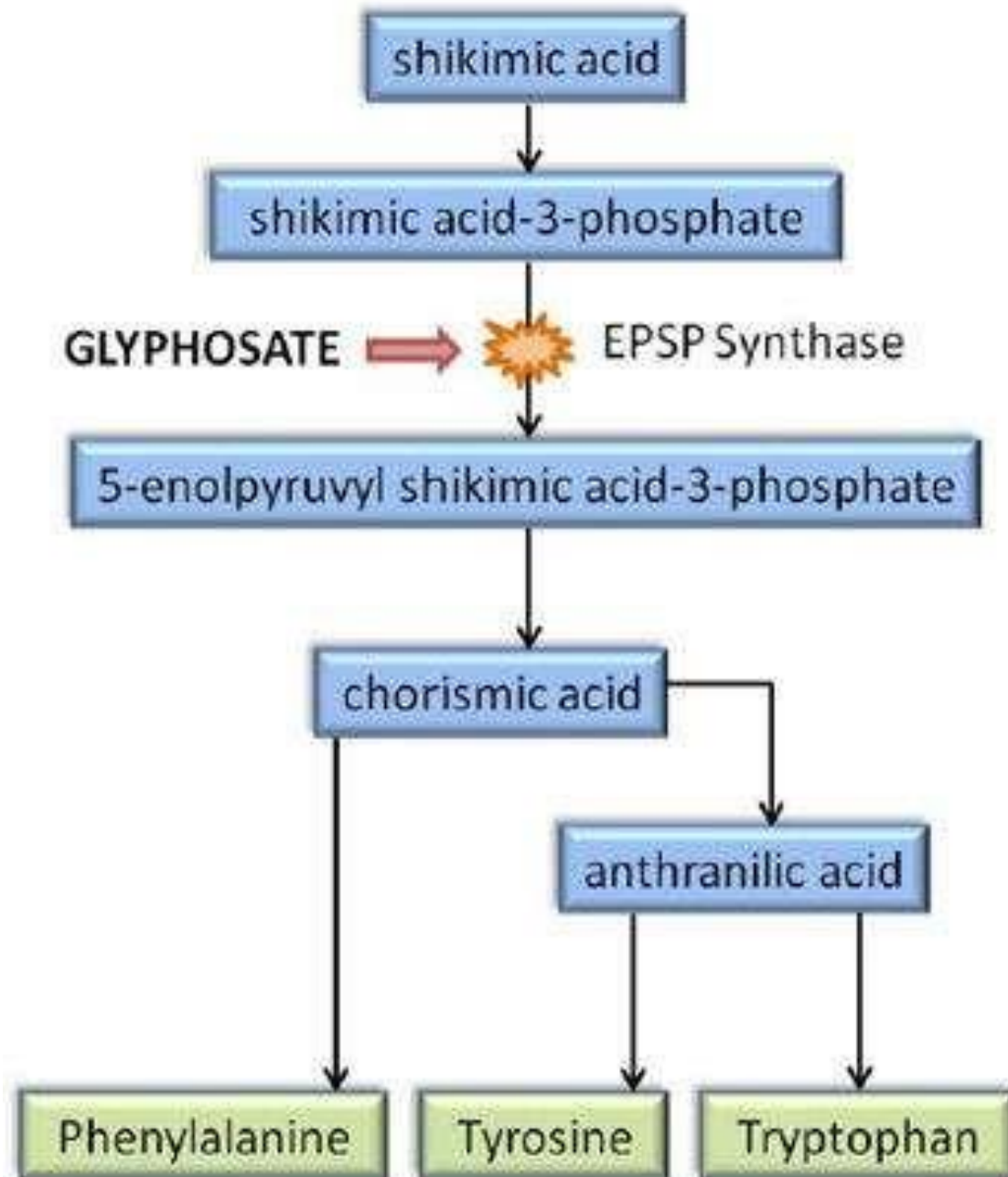
Roundup Ready Soybean

- *In 1994 the first genetically modified soybean was introduced to the U.S. market, by **Monsanto***
- *In 2014, 90.7 million hectares of GM soy were planted worldwide, 82% of the total soy cultivation area*
- *Roundup Ready Soybeans are a series of genetically engineered varieties of glyphosate-resistant soybeans produced by **Monsanto***



Glyphosate (A Herbicide)

- Also known as **Roundup**
- Glyphosate kills plants by interfering with the synthesis of the essential amino acids **phenylalanine, tyrosine and tryptophan**
- These amino acids are called "essential" because animals cannot make them; only plants and micro-organisms can make them and animals obtain them by eating plants
- Plants and microorganisms make these amino acids with an enzyme called **5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)**
- At the molecular level, Glyphosate inhibits this enzymes activity



Mechanism of Resistance

- express a version of EPSPS from the CP4 strain of the bacteria, *Agrobacterium tumefaciens*
- expression of which is regulated by an enhanced 35S promoter (E35S) from cauliflower mosaic virus (CaMV), a chloroplast transit peptide (CTP4) coding sequence from *Petunia hybrida*, and a nopaline synthase (nos 3') transcriptional termination element from *Agrobacterium tumefaciens*
- The plasmid with EPSPS and the other genetic elements mentioned above was inserted into soybean germplasm with a gene gun by scientists at Monsanto and Asgrow

Thank you

