PLNT2530 (2024)
Unit 10b

Applications of Plant Biotechnology in Agriculture

Insect Resistance

Plant Biotechnology

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Chapters 5-10

Insect pest control

- Insect pest damage can be quite extensive (crop or seed)

- Major damage is normally a result of insect larva feeding

- Major insect pests fall into several groups
    - Lepidoptera ex. European corn borer, cotton bollworm
    - Coleoptera ex. Colorado potato beetle, confused flour beetle
    - Orthoptera ex Locust, crickets, grasshoppers
    - Homoptera ex Aphids
    - Diptera ex. Mosquitoes and flies

Virtually all insecticides are toxic to animals, including humans! They require careful handling protocols as hazardous materials

Bacillus thuringensis toxin (Bt)
"Pesticidal crystal protein"

- Discovered in 1901 as affecting silkworms

- Bacteria produces protein crystals as inclusion bodies during sporulation. These proteins if ingested by insects were shown to have insecticidal properties and were designated delta-endotoxins

- These toxins are part of a super-family of genes with at least 51 distinct families based on sequence differences Referred to as the *cry genes (cry1- cry51)* and the products as Cry proteins or Bt toxins
- Cry genes are plasmid-encoded in *B. thurngiensis*

Bacillus thuringensis (Bt)

- 4 Major classes of Bt protein based on insect sensitivity to toxin
  - Cry1 Lepidoptera
  - Cry2 Lepidoptera and Diptera
  - Cry3 Coleoptera
  - Cry4 Diptera

They are further arranged into subclasses (A,B,C..) and subgroups (a,b,c,…) based on the DNA sequence of the toxin genes

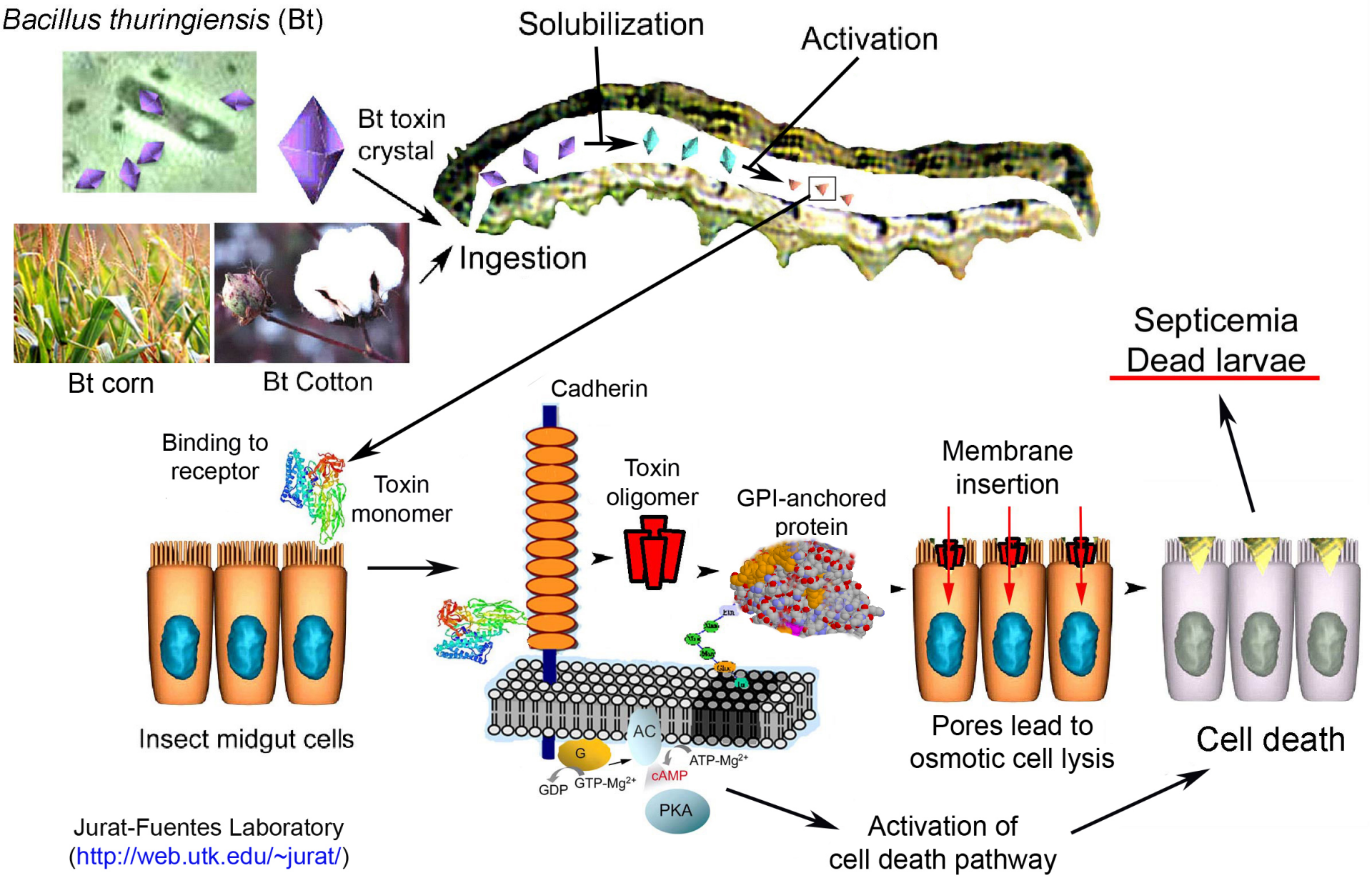
- Different strains of Bt produce different toxins

- Bt spores have been used for >50 y as an organic pesticide

Bt toxins mode of action

- Normally insects encounter the toxins by ingesting the Bt spores.
- Limited proteolytic cleavage of the Cry proprotein in the gut converts the inactive toxin into an active form which binds to a high affinity receptor in the insect midgut brush-border membrane
- Results in opening a cation selective pore in the epithelial cell membrane – influx of ions and osmotic lysis of the epithelial cell.
- Destruction of this absorption surface is lethal to the insect
- Differential sensitivity of insect classes to the various toxins, and animal and human insensitivity made the Bt toxins attractive as potential bioinsecticides

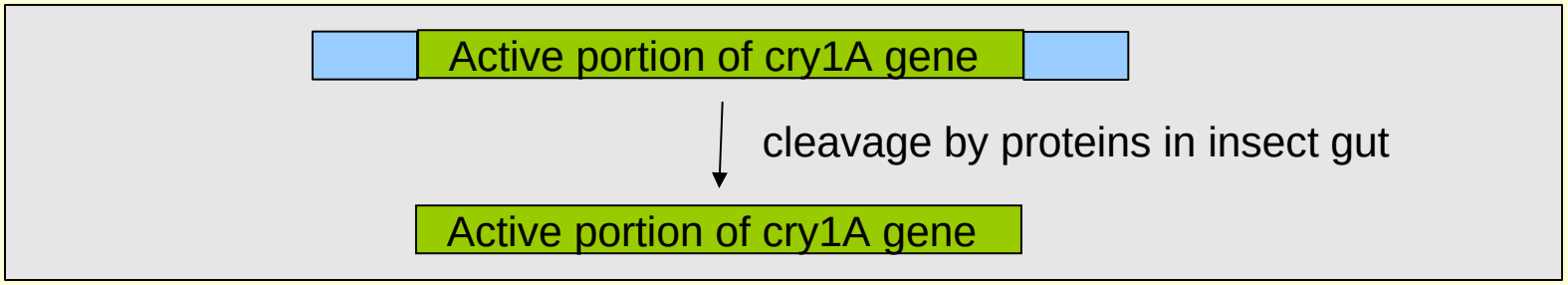
Bt toxins mode of action



Jurat-Fuentes Laboratory
(http://web.utk.edu/~jurat/)

Development of Bt protection in crop plants

- Protoxins vary in size but when cleaved will release a toxin of 550-700 amino acids long - active region used for transgene



construct: CaMV 35S | Active portion of cry1A gene | NOS 3'

		Bt as % of total leaf protein	Insecticidal activity
Initial construct	original cry1A gene	0.003-0.012	+
Version 1	Partial codon modification	0.002- 0.02	++
Version 2	Complete codon modification	up to 0.3	+++

Original Bt *cry* gene was 37% G+C while target plant genes were 49% G+C

Commercial Bt products

Company	Trade name	Bt protein	Crop	Insect pest
Monsanto	New leaf*	Cry3A	potato	Colorado beetle
	Bollgard	Cry1Ac	cotton	Cotton bollworm
	YieldGard	Cry1Ab	maize	European corn borer
		Cry3Bb	maize	Corn rootworm larvae
DeKalb	Bt-Xtra	Cry1Ac	maize	European corn borer
Aventis	StarLink	Cry1C	maize	European corn borer
Mycogen	Herulex 1	Cry1F	maize	European corn borer

Note: Cotton is a very insecticide-intensive crop. Conventional cotton is therefore one of the most polluting and hazardous crops to work with. Bt cotton could therefore be considered an example of a GM crop being more environmentally friendly than the conventional crop.

* Discontinued due to public pressure

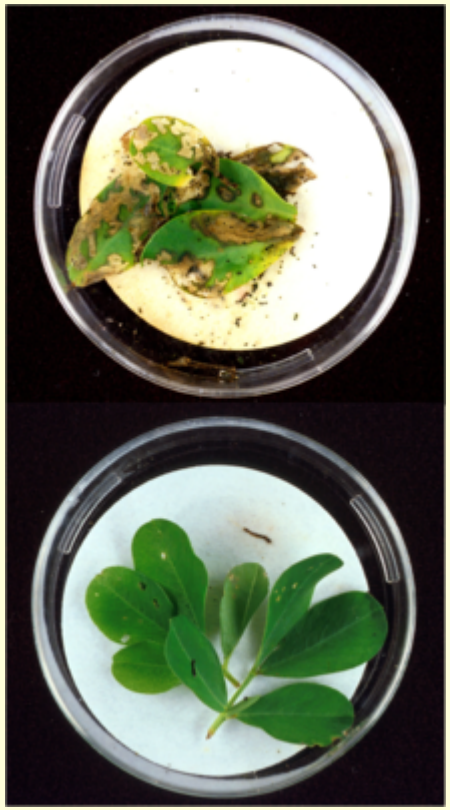
Cotton bollworm

Bt cotton

Wild type cotton

Lesser cornstalk borer

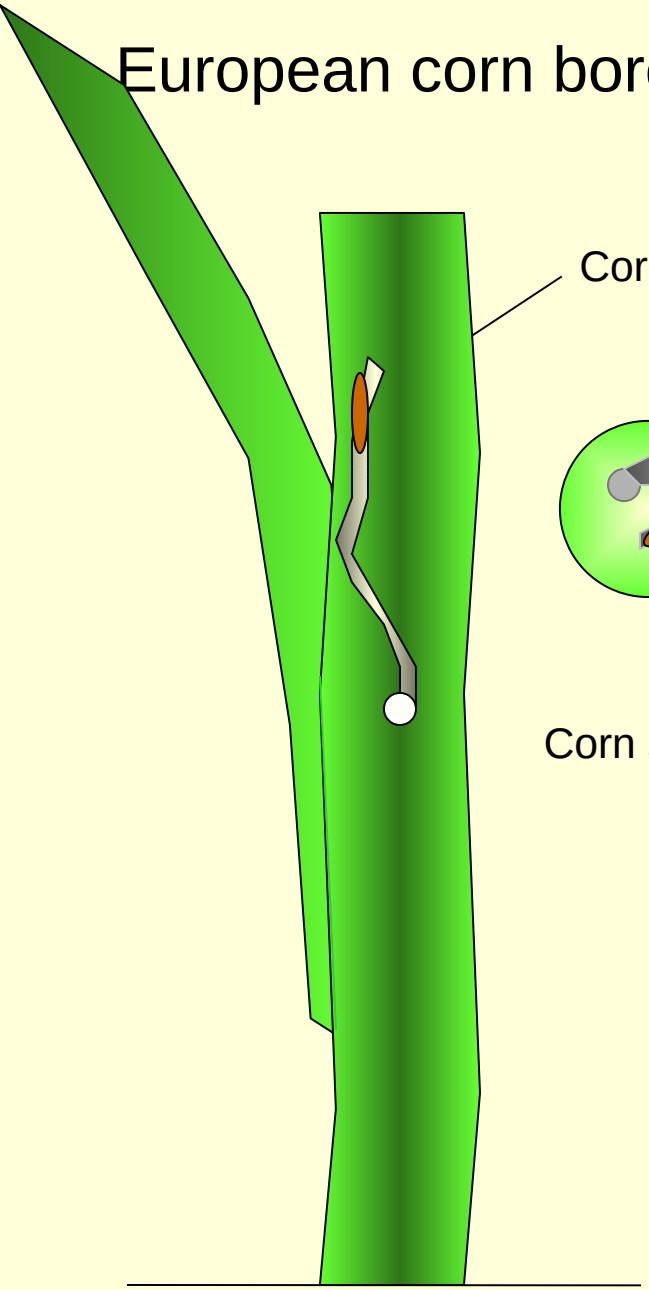
Wild type Peanut



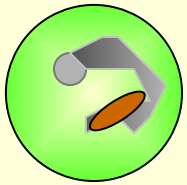
Bt Peanut

http://agresearchmag.ars.usda.gov/1999/nov/pest/

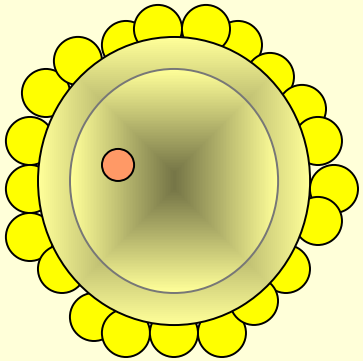
European corn borer damage



Corn borer tunnels inside the stalk and cobs – seldom exposed to insecticides



Corn stalk



Cob cross section

Internal location of the corn borer makes it difficult to control with insecticides

Use of Bt corn has significantly reduced insecticide use in corn crops

Note: 35S promoter doesn't work in monocots
Used ubiquitin promoter instead.

Issues with Bt pest control

- High use of Bt has shown the development of pest resistance

- Resistant insects arise by <u>mutations in the membrane epithelial binding protein</u> – result: - non-binding of the Bt toxins

- To slow the development of resistance the recommended strategy is the maintenance of a population of susceptible insects. (*refugia*)

- Resistance, in the form of midgut receptors that don't bind Bt, should be recessive. Homozygous resistant (bb) insects will breed with susceptible insects. The heterozygous insect (Bb) should still be sensitive to Bt, since it still produces active receptor proteins.

More on Refugia

Refuges of genetic variation: controlling crop pest evolution

http://evolution.berkeley.edu/evolibrary/article/agriculture_04

More on Refugia

Some Bt crops are sold as a mixture of conventional and Bt. This makes the entire field a refuge. Mixtures vary, but a common ratio is 75% Bt, 25% conventional.

Mix Is Key in Reversing Pest Resistance to Biotech Cotton
https://news.arizona.edu/story/mix-key-reversing-pest-resistance-biotech-cotton

This strategy is widely used in Bt cotton

Advantages of Bt

- Moderately host-specific to classes of pest
- More friendly to Monarch Butterflies and other tolerant species normally found in crops that would otherwise be sprayed with non-specific insecticide
    - Monarch butterflies are sensitive to the same Cry toxins as are European corn borers. However monarchs feed primarily on milkweed growing in corn fields, rather than corn itself.
    - In principle, Monarchs might be affected on those days when corn is pollinating, if pollen falls on milkweed. The solution was to replace ubiquitin promoter with PEP carboxylase promoter, which is expressed only in green tissue.
      http://passel.unl.edu/pages/informationmodule.php?idinformationmodule=959031259&topicorder=4&maxto=9