Microbial pesticides

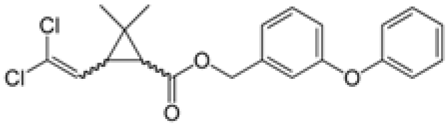
Food security and challenges

- Feeding 9 billion by 2050
- Global demand for food- 70 % by 2050
- 30 % loss of stored food (Boyer *et al.*, 2011)
- More than 3 million people are killed- malaria (Ansari et al., 2014)

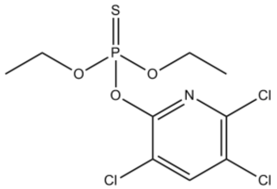
- *Bemisia tabaci* (whitefly)
- *Aphis gossypii* (Cotton aphid)

- *Helicoverpa armigera* (cotton bollworm)
- Changes in climatic conditions, pest re-emergence, - threatening agricultural production (Sundstrom *et al.*, 2014)

Insecticide resistance



Pyrethroid

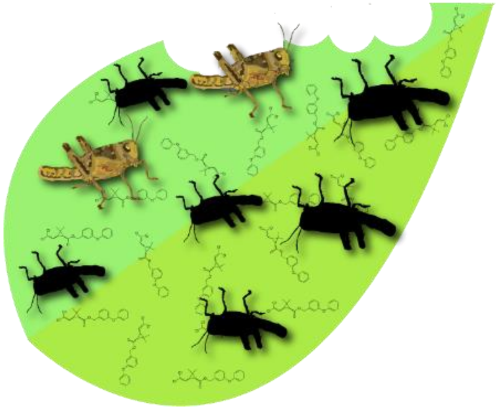


Organophosphate

Pesticide usage

4.6 million tonnes of pesticides

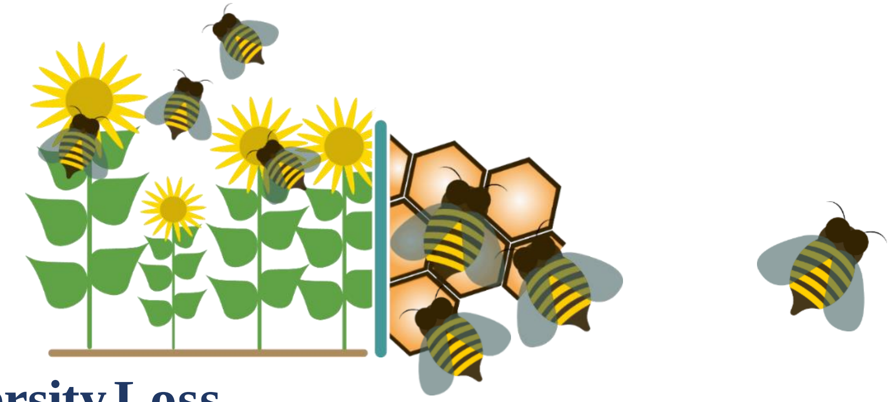
(Ansari *et al.*, 2014)



Insect resistance to pesticides

- *B. tabaci*- pyrethroid- *Vgsc* and *Ace* gene (Gauthier *et al.*, 2014)
- *Spodoptera litura*- profenofos- *Ace* gene (Su *et al.*, 2014)

Effects on Non-target organisms



Biodiversity Loss
1 in 3 crops pollination

Colony Collapse Disorder

- Imbalance in terrestrial ecosystem stability
- Crop productivity
- Human welfare

Environmental problem

Insect resistance to insecticides

- Targets- Acetylcholine esterase, Voltage Gated Channels, Hormones (Hardy, 2014)
- Pyrethroids, organophosphates, DDT
- Globally, 4.6 million tonnes of pesticides (Ansari *et al*., 2014)
- Mechanisms- detoxification by cytochrome P450, mutations in targeted sites (Zimmer et al., 2014)
  - *B. tabaci*- pyrethroid- *Vgsc* and *Ace* gene (Gauthier *et al*., 2014)
  - *Spodoptera litura*- profenofos- *Ace* gene (Su *et al*., 2014)
  - *Anopheles gambiae sensu lato* - deltamethrin and DDT (Nkya *et al*., 2014)

Environmental and human health risks

- Effects on non target organisms
- Pollinators- Biodiversity maintainer
- 71 of 100 crops are pollinated by bees
- Imbalance in terrestrial ecosystem stability → Crop productivity → Human welfare
  - Imidacloprid- Field level exposure- reduced colony growth and 85 % reduction in Queen Bee production (Whitehorn *et al.*, 2012)
- 3 million cases of pesticide poisoning each year; up to 2,20,000 deaths, primarily in developing countries (WHO report)

Biopesticides

- "Any molecules from the biological origin, whole organism or product derived from them" (Villaverde *et al.*, 2014)
- **Bacteria**
  - *Bacillus thuringiensis* (*Bt*)- Cry, Cyt, Vip
  - *Ostrinia nubilalis*- major pest of corn (Crava *et al.*, 2014), *S. exigua* (Beet army worm) (Naimov *et al.*, 2014)
- **Virus**
  - Baculovirus- Itch mite toxin Txp-1- Increased mortality to 50-60% (Burden *et al.*, 2000)
- **Fungi**
  - *Lecanicillium lecanii , Isaria fumosorosea, Beauveria, and Metarhizium*

Biological pesticides

"Any molecules from the biological origin, whole organism or product derived from them" (Villaverde *et al.*, 2014)

Bacteria

- *Bacillus thuringiensis* (*Bt*)

  Cry, Cyt, Vip

Virus

- Baculovirus

Needs to be ingested to act

Fungi

- ***Beauveria, Metarhizium,***
- *Lecanicillium lecanii*
- *Isaria fumosorosea,*

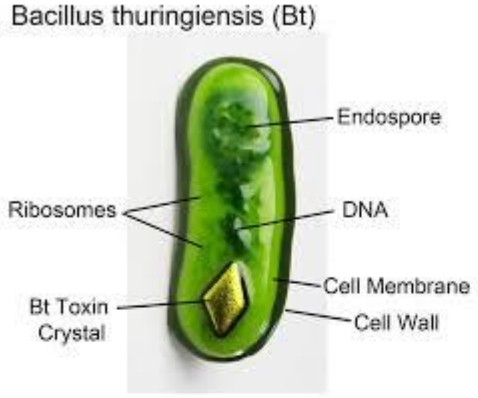
Acts by direct penetration (or)

Contact infection

Bacillus thuringiensis

Bacillus thuringiensis

- ***Bacillus thuringiensis*** (or **Bt**) is a Gram-positive, soil-dwelling bacterium, commonly used as a biological pesticide.
- *B. thuringiensis* also occurs naturally in the gut of caterpillars of various types of moths and butterflies.
- During sporulation, many Bt strains produce crystal proteins (proteinaceous inclusions), called δ-endotoxins, that have insecticidal action.
- This has led to their use as insecticides, and more recently to genetically modified crops using Bt genes, such as Bt corn.
- The subspecies *israelensis* is commonly used for control of mosquitoes



Bacillus thuringiensis

- *Bt* was isolated in 1901 and named in 1911. It was used as a commercial biopesticide for the first time in the United States in 1958.
- **Delta endotoxins** (**δ-endotoxins**) are pore-forming toxins produced by *Bacillus thuringiensis* species of bacteria.
- During spore formation the bacteria produce crystals of such proteins (hence the name **Cry** toxins) that are also known as **parasporal bodies**, next to the endospores; as a result some members are known as a **parasporin**.
- The **Cyt** (cytolytic) toxin group is a group of delta-endotoxins different from the Cry group.
- The activated region of the delta toxin is composed of three distinct structural domains: an N-terminal helical bundle domain involved in membrane insertion and pore formation; a beta-sheet central domain involved in receptor binding; and a C-terminal beta-sandwich domain that interacts with the N-terminal domain to form a channel

Bt Toxin Nomenclature:

Each Bt toxin will be assigned a unique name incorporating four ranks
e.g. Cry 1Aa3

- Primary rank - order of insect;
- Secondary and tertiary ranks - potency and targeting within an order
- Quaternary rank- alleles of genes coding for toxins

- Classification based on their sequence homology and specificities

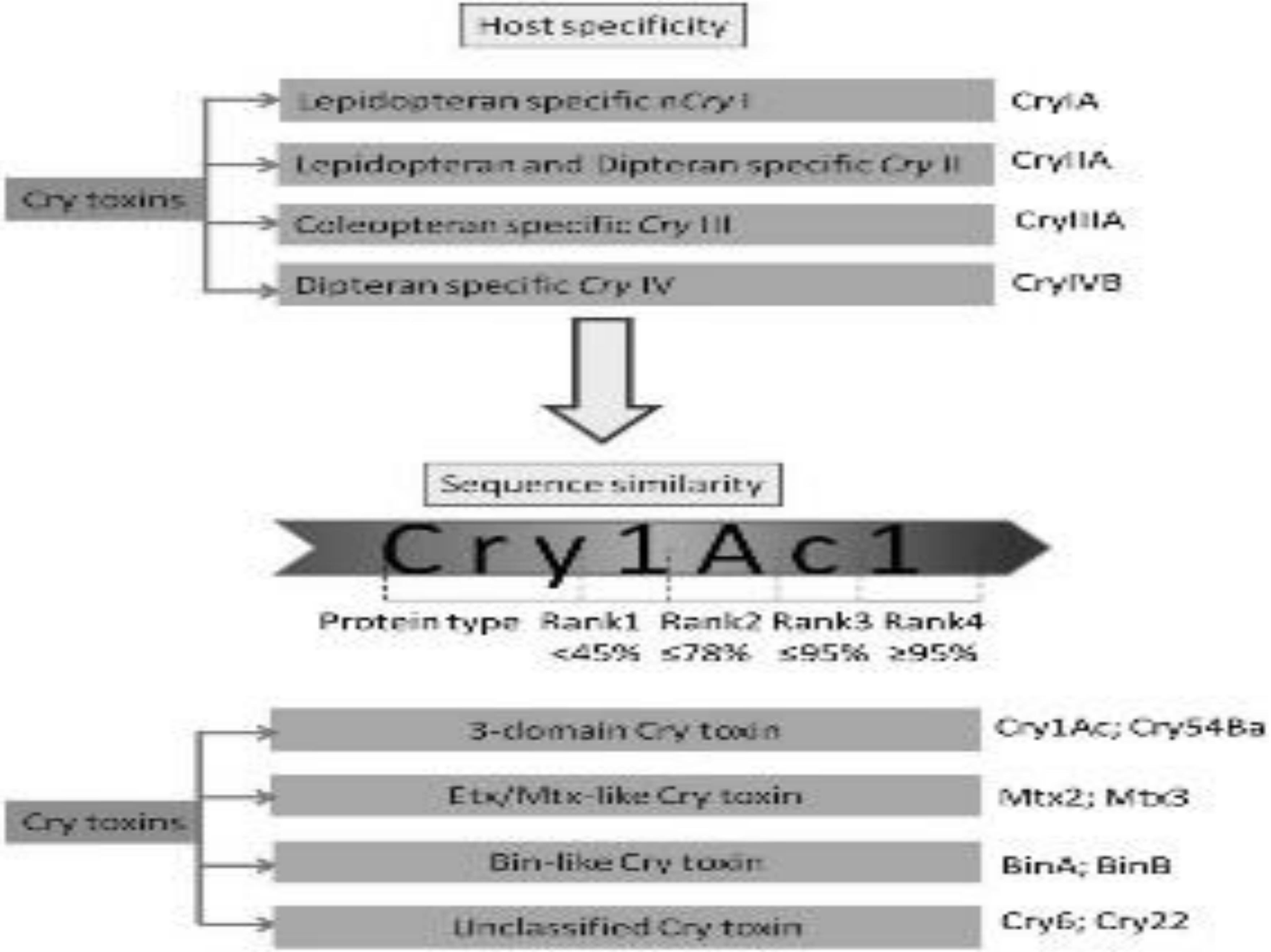
- CryI genes encoded proteins toxic to lepidopterans;

- CryII genes encoded proteins toxic to both lepidopterans and dipterans;

- CryIII genes encoded proteins toxic to coleopterans;

- CryIV genes encoded proteins toxic to dipterans alone.

Crickmore , 1998



Host specificity
Cry toxins
Lepidopteran specific Cry I
CryIA
Lepidopteran and Dipteran specific Cry II
CryIIA
Coleopteran specific Cry III
CryIIIA
Dipteran specific Cry IV
CryIVB
Sequence similarity
Cry1Ac1
Protein type
Rank1 <45%
Rank2 ≤78%
Rank3 ≤95%
Rank4 ≥95%
Cry toxins
3-domain Cry toxin
Cry1Ac; Cry54Ba
Etx/Mtx-like Cry toxin
Mtx2; Mtx3
Bin-like Cry toxin
BinA; BinB
Unclassified Cry toxin
Cry6; Cry22

Mode of action:

- When insects ingest toxin crystals, their alkaline digestive tracts denature the insoluble crystals, making them soluble and thus amenable to being cut with proteases found in the insect gut, which liberate the toxin from the crystal.
- The Cry toxin is then inserted into the insect gut cell membrane, paralyzing the digestive tract and forming a pore.
- The insect stops eating and starves to death; live Bt bacteria may also colonize the insect, which can contribute to death.
- Death occurs within a few hours or weeks. The midgut bacteria of susceptible larvae may be required for *B. thuringiensis* insecticidal activity.

Mode of action:

Stage I – Proteolysis of protoxins in the insect gut to generate active toxin fragments

The midgut pH and proteolytic enzymes present in different insect orders – determinants of the selective toxicity of particular Cry protoxins.

Lepidoptera – Highly alkaline (9.5-10.5), proteases present are typsins and chymotrypsins.

Coleoptera – pH range is 5.5 to 8.0 and aspartic and cysteine proteases

Stage II- Binding of Cry toxins to specific receptors on midgut epithelial cells

- *Bt* action is very specific. Different strains of *Bt* are specific to different receptors in insect gut wall.
- *Bt* toxicity depends on recognizing receptors, damage to the gut by the toxin occurs upon binding to a receptor.
- Each insect species possesses different types of receptors that will match only certain toxin proteins, like a lock to a key.

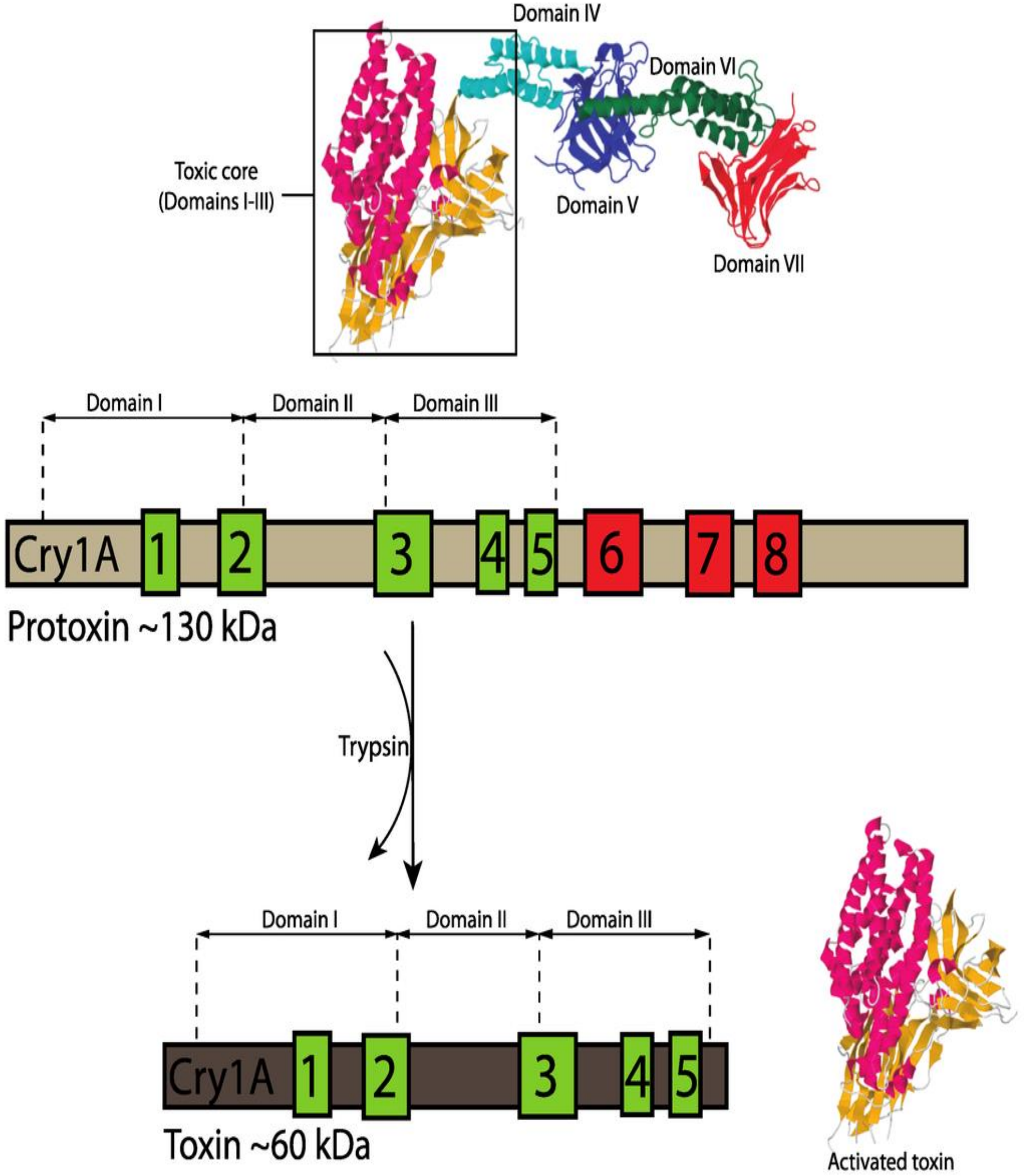
Mode of action:

Stage III – Formation of transmembrane pores

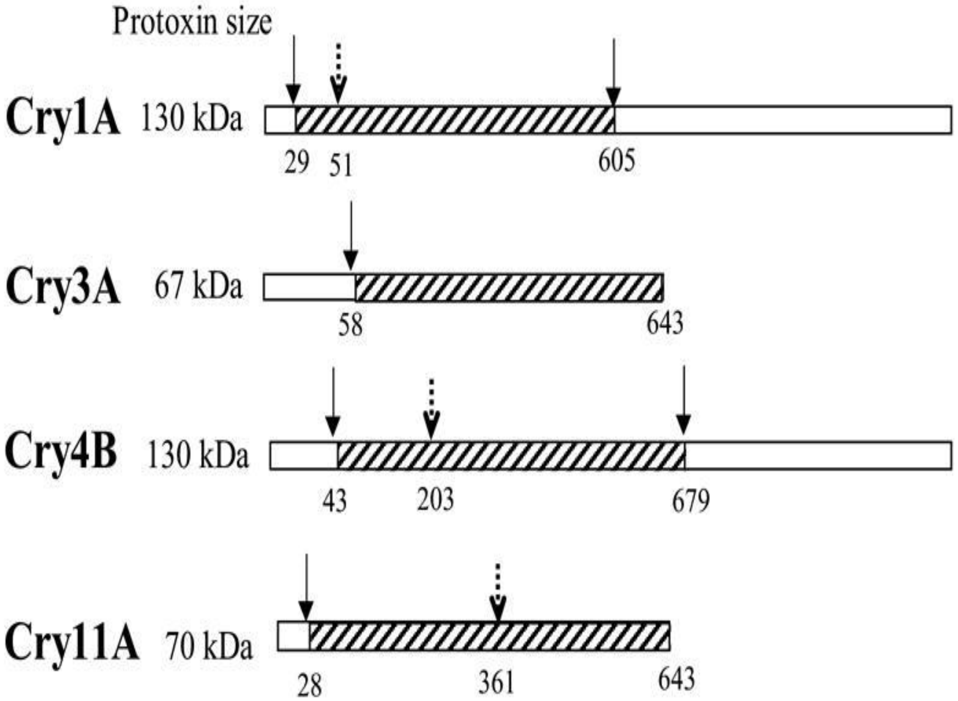
- Binding leads – toxin gets localized at the external surface of the cytoplasmic membrane of epithelial cells of the midgut brush border membrane and binding induces a conformation change in the toxin which is necessary for membrane insertion
- Upon insertion the toxin oligomerizes and forms transmembrane cation selective pores
- The pores allow equilibration of cation concentrations across the membrane. The entry of Na^+ ions with an accompanying influx of water leads to swelling and rupture of the cell wall.

Stage IV – Bacteremia/Septicimia

- The larvae stops feeding and ultimately dies. The vegetative cells of Bt that germinate from the ingested spores are able to enter the haemolymph through the damaged epithelial cell layer and multiply.

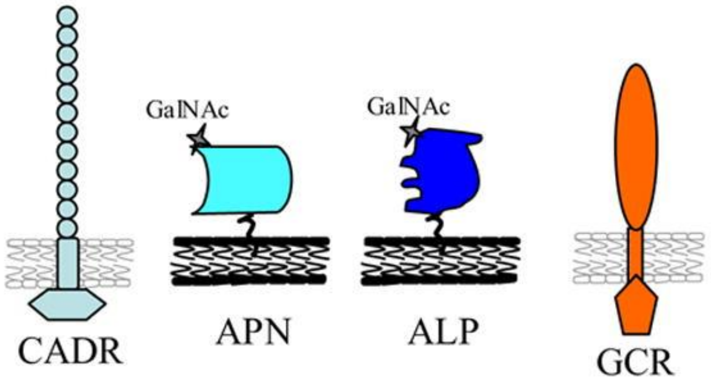


Domain IV
Domain VI
Toxic core
(Domains I-III)
Domain V
Domain VII
Domain I
Domain II
Domain III
Cry1A
1
2
3
4
5
6
7
8
Protoxin ~130 kDa
Trypsin
Domain I
Domain II
Domain III
Cry1A
1
2
3
4
5
Toxin ~60 kDa
Activated toxin



Protoxin size
Cry1A 130 kDa
29 51 605
Cry3A 67 kDa
58 643
Cry4B 130 kDa
43 203 679
Cry11A 70 kDa
28 361 643

Receptors



Receptor molecules of Cry1A proteins. CADR, cadherin receptor; APN, aminopeptidase-N, ALP, alkaline phosphatase, GCR, 270 kDa glyco-conjugate receptor.

Bt action is very specific. Different strains of *Bt* are specific to different receptors in insect gut wall.
Bt toxicity depends on recognizing receptors, damage to the gut by the toxin occurs upon binding to a receptor.
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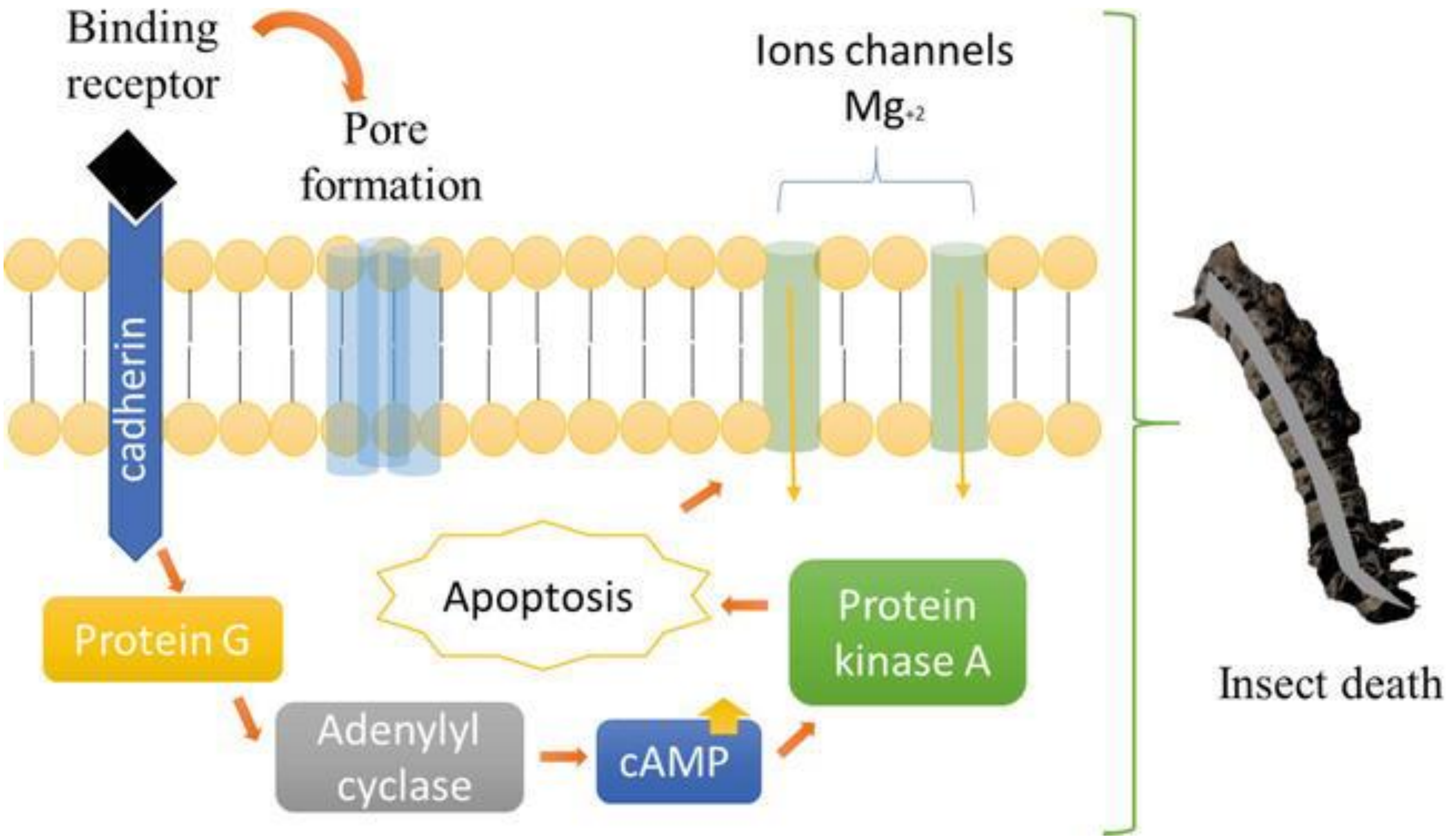
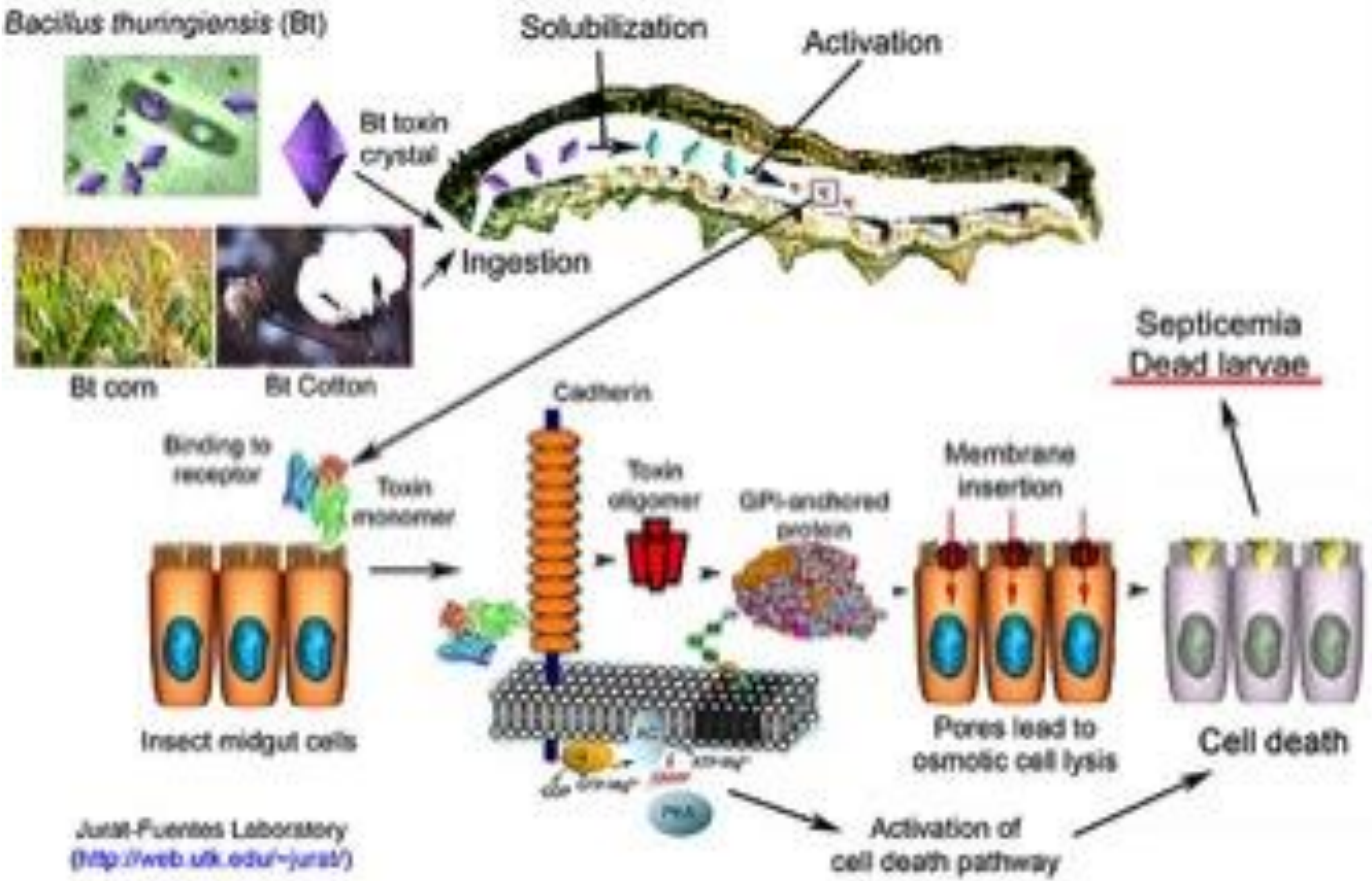
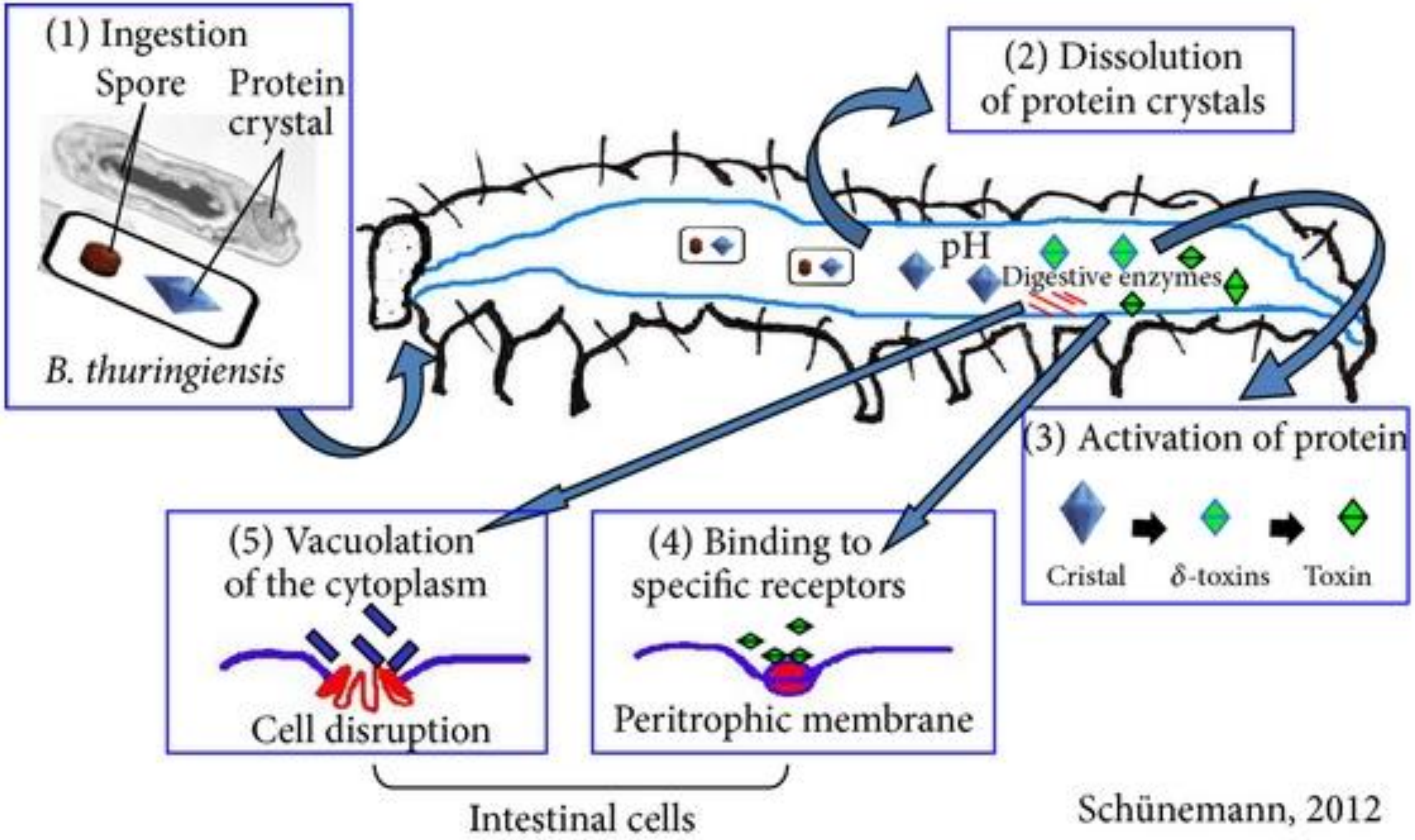

Binding
receptor
Pore
formation
Ions channels
Mg+2
cadherin
Protein G
Apoptosis
Protein
kinase A
Adenylyl
cyclase
cAMP
Insect death


Bacillus thuringiensis (Bt)
Bt toxin crystal
Solubilization
Activation
Ingestion
Bt corn
Bt Cotton
Septicemia
Dead larvae
Cadherin
Binding to receptor
Toxin monomer
Toxin oligomer
GPI-anchored protein
Membrane insertion
Insect midgut cells
Pores lead to osmotic cell lysis
Cell death
Activation of cell death pathway
Jurat-Fuentes Laboratory
(http://web.utk.edu/~jurat/)



(1) Ingestion
Spore
Protein crystal
B. thuringiensis
(2) Dissolution of protein crystals
pH
Digestive enzymes
(3) Activation of protein
Cristal
δ-toxins
Toxin
(4) Binding to specific receptors
Peritrophic membrane
(5) Vacuolation of the cytoplasm
Cell disruption
Intestinal cells
Schünemann, 2012