How to Develop an Insect Resistance Management Plan:
Practical Approaches for Local Environments

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Today's Topics

- Global Adoption of Agriculture Biotechnology
  – Characteristics of insect control traits
- Why is Insect Resistance Management (IRM) Important for Transgenic Insecticidal Crops?
  – Scientific theory of IRM strategy
  – Definitions & purpose
  – Foundation and tactics

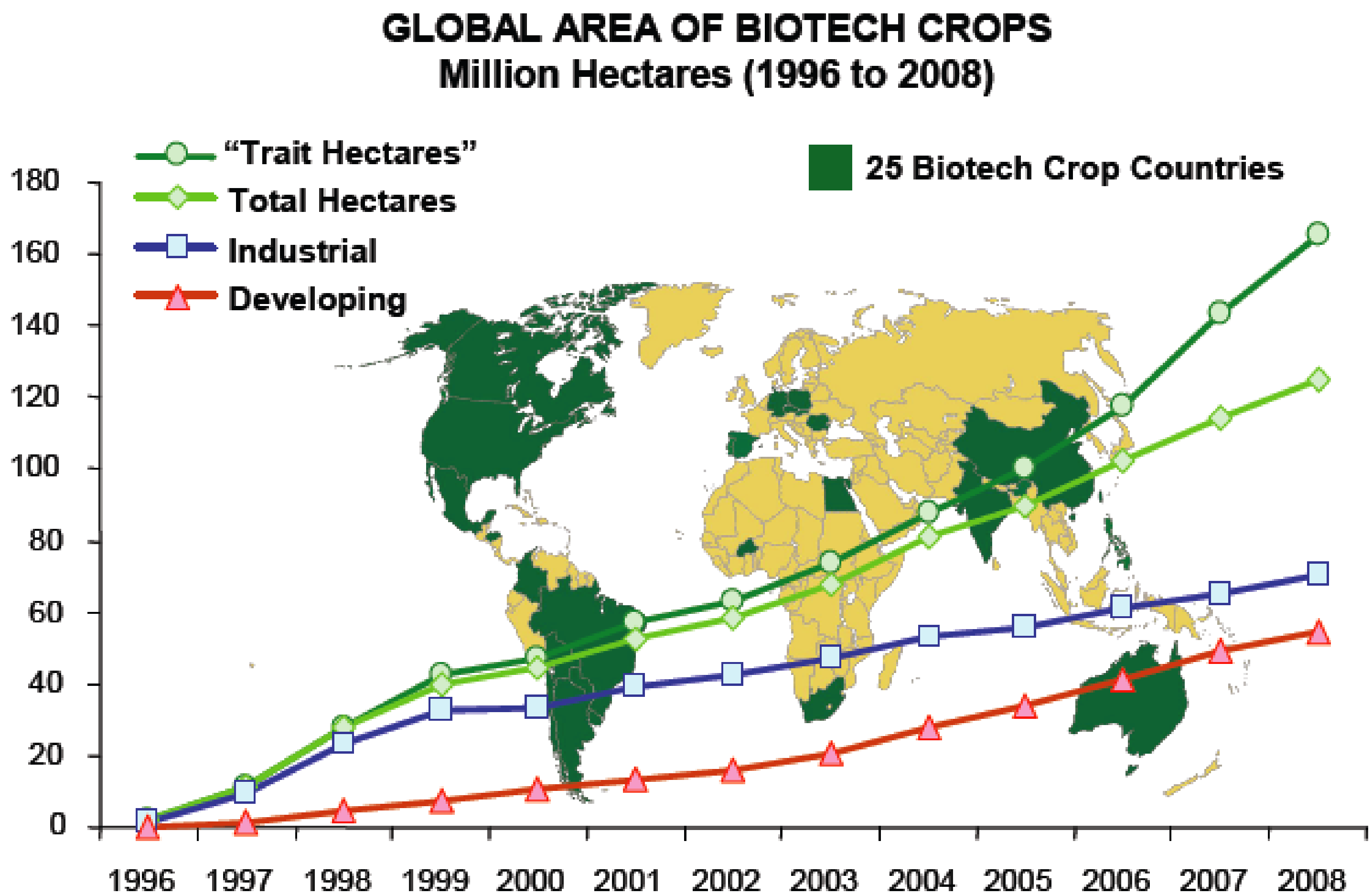
Today's Topics

- USA Experience with Insect Resistance Management
  - Maize, Pyramiding Insecticidal Proteins, Cotton
- Global Experience with IRM
- How to Develop an IRM Plan

Biotechnology in Agriculture

- Biotechnology has been enthusiastically embraced by growers around the world
  - More than 125M Ha were planted in 2007
  - Grown by 13.3M growers in 25 different countries
  - 90% of these growers are in developing nations
  - The fastest growing regions are the developing nations

Global Distribution of Biotech Crops



An "apparent" increase of 9.4% or 10.7 million hectares between 2007 and 2008, equivalent to a "real" increase of 15% or 22 million "trait hectares"

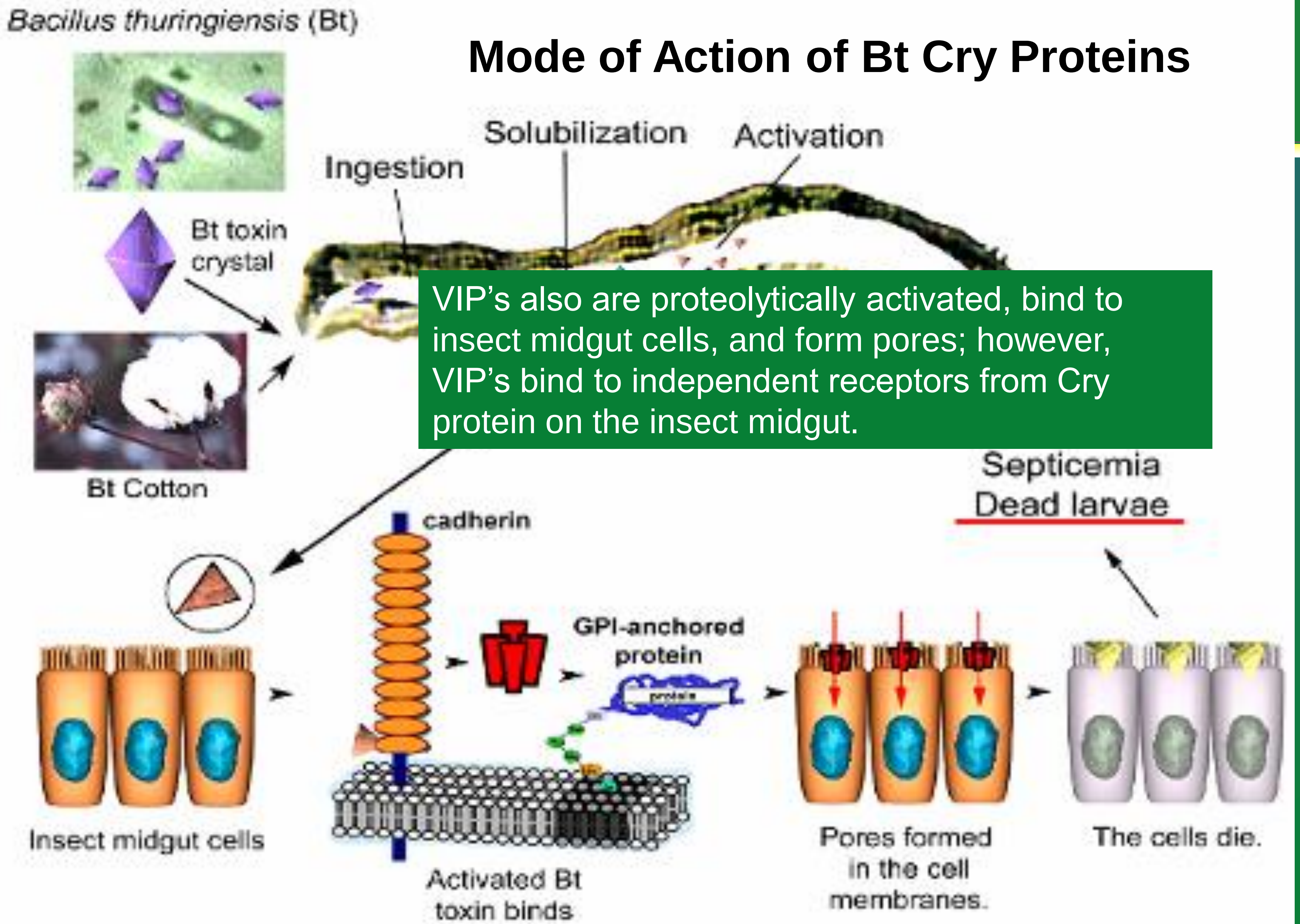
Source: Clive James, 2009.

Insect Control Traits

- While most of these biotech hectares are planted with herbicide tolerant traits, about 33% of the hectares (>40M Ha) are planted with insect control traits, *Bt* and/or VIP
- *Bacillus thuringiensis* (*Bt*) a natural bacterium that produces insect control proteins, called Cry and VIP proteins
  - Cry = crystal proteins because they form crystals in the native bacterium and control coleopteran or lepidopteran crop pests
  - VIP = Vegetative insecticidal proteins are secreted during the vegetative bacterial stages prior sporulation

Cry and VIP proteins have superior environmental and health benefits!

Mode of Action of Bt Cry Proteins



VIP's also are proteolytically activated, bind to insect midgut cells, and form pores; however, VIP's bind to independent receptors from Cry protein on the insect midgut.

Resistance – What is it?

- Resistance is defined as the ability of an organism to 'defend' itself from disease.
  - In agriculture, resistance has a slightly different meaning, when a pest can defend itself from the controlling agent:
    - ✓ When weeds are able to survive an herbicide
    - ✓ When insects survive an insecticide
- When insect populations develop resistance, it is typically a gradual process where they are able to withstand higher and higher amounts of insecticide, until finally, the insecticide is no longer effective in controlling the insect pest.

Not all insects develop resistance, pest biology and insecticide selection pressure influence the result

Insect Resistance Management

The greatest risks to Bt and VIP crops is the potential to develop insect resistance: Long-term product durability is the goal!

- **Resistance has occurred with sprayable *Bt* sprayable formulations since the mid-1990s**
  - **Mostly Diamondback moth populations in tropical regions**
- **Highly effective insect control with *Bt* and VIP crops has led to concerns of insect resistance evolution**

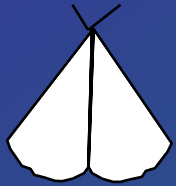
- Utilize IRM knowledge and experience: >10 years of Bt crop success
  - Argentina, Australia, Canada, China, Philippines, United States

BUT IRM plans must be tailored to local or regional environments!

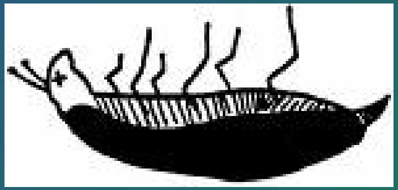
Resistance Management Basics

Definitions:

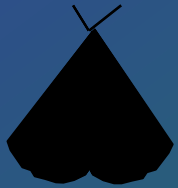
- **Susceptible insect = one readily killed by the toxin**



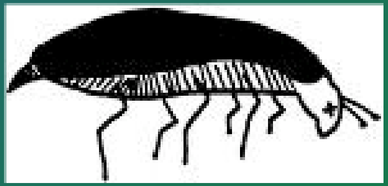
susceptible insect + *Bt* or VIP =



- **Resistant insect = one that survives the toxin**

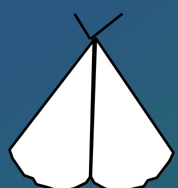
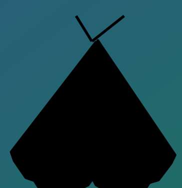


resistant insect + *Bt* or VIP =



Definitions: Genetic Traits

- **Allele = a pair of genes that cause a trait**
- **Genotype = the genetic make-up of an organism, the genotype cannot be seen**
- **Phenotype = the observable trait or properties of an organism**

Phenotype		Genotype
	susceptible insect	= s / s Alleles
	resistant insect	= r / r Alleles

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Definitions: Genetic Dominance

- **Alleles can have different 'strengths', meaning that they can be dominant or recessive in their effect on the trait phenotype**
- **Dominant = a gene combination that effects the phenotype of an organism with a single allele**
- **Recessive = a genetic make-up that has no impact on the phenotype unless it present in both allele.**

Trait Dominance: Examples

	Phenotype		Genotype	Trait Dominance
Recessive allele				
	susceptible insect	=	s / s Alleles	Unknown
	susceptible insect	=	r / s Alleles	Recessive
	resistant insect	=	r / r Alleles	Recessive
Dominant allele				
	susceptible insect	=	s / s Alleles	Unknown
	resistant insect	=	s / R Alleles	Dominant
	resistant insect	=	R / R Alleles	Dominant

s = susceptible allele, r = recessive resistance allele, R = dominant resistance allele

Definition: Dose

- **Dose = the amount of insecticidal protein in the plant:**
  - High dose kills all target insects and all heterozygous insects (offspring of resistant and susceptible insects)
  - Medium dose kills close to 100% of the susceptible insects
  - Low dose kills significantly less than 100% of the susceptible insect

Genetic Dominance & Dose

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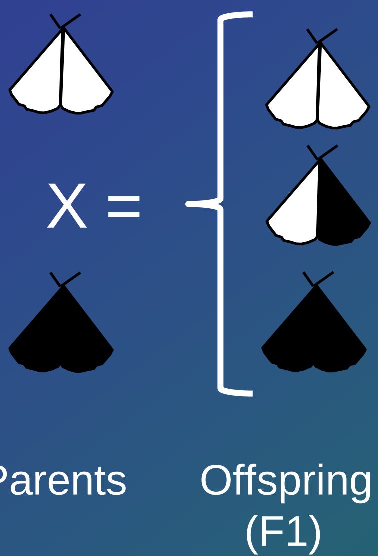


If the trait is:

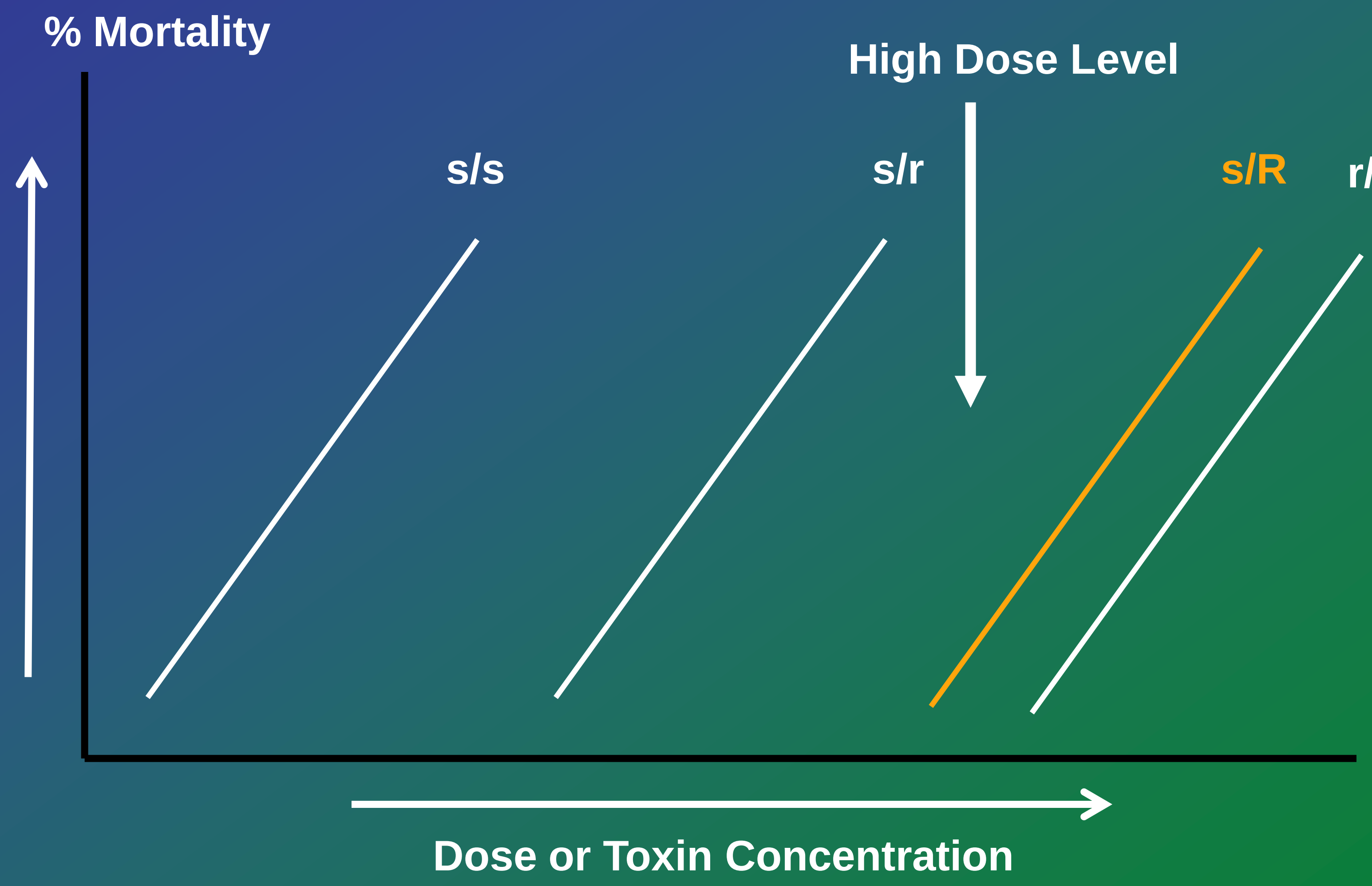
Recessive OR **Dominant**

Population percentage	Recessive: Phenotype	Recessive: Dose	Dominant: Phenotype	Dominant: Dose
25%	Susceptible	Med/High	Susceptible	Med/High
50%	Susceptible	High	Resistant	—
25%	Resistant	—	Resistant	—

What part of the population was controlled?

	Recessive	Dominant
What part of the population was controlled?	75%	25%

Genetic Dominance and Dose



Dose & Susceptibility

As larvae grow, they become less sensitive to the insecticidal protein and will cause more feeding damage This effect is unrelated to resistance.

Resistance Management Basics

Purpose:

An Insect Resistance Management (IRM) Plan utilizes various activities to minimize selection pressure on the target pests. IRM reduces pest populations by as many different tactics as possible to maintain the ability to control the target pests: Analogous to Integrated Pest Management. Other aspects communicate the plan to stakeholders, monitor the effectiveness of the IRM plan and ensure compliance with it’s elements.

To extend the product durability for as long as possible

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Foundation of an IRM Plan

- The biology of the crop, pest and crop/pest interaction is the foundation of an IRM plan
  - Crop biology will differ according to the growing region
  - The pest spectrum will also vary
  - The insect movement patterns of both larvae and adults will be unique for each crop
- The target pest susceptibility to the Cry or VIP protein is determined to shape the IRM plan
  - Is the expression level in the insecticidal crop sufficient to control the pest(s)?
  - Is the level considered low, medium or high dose?

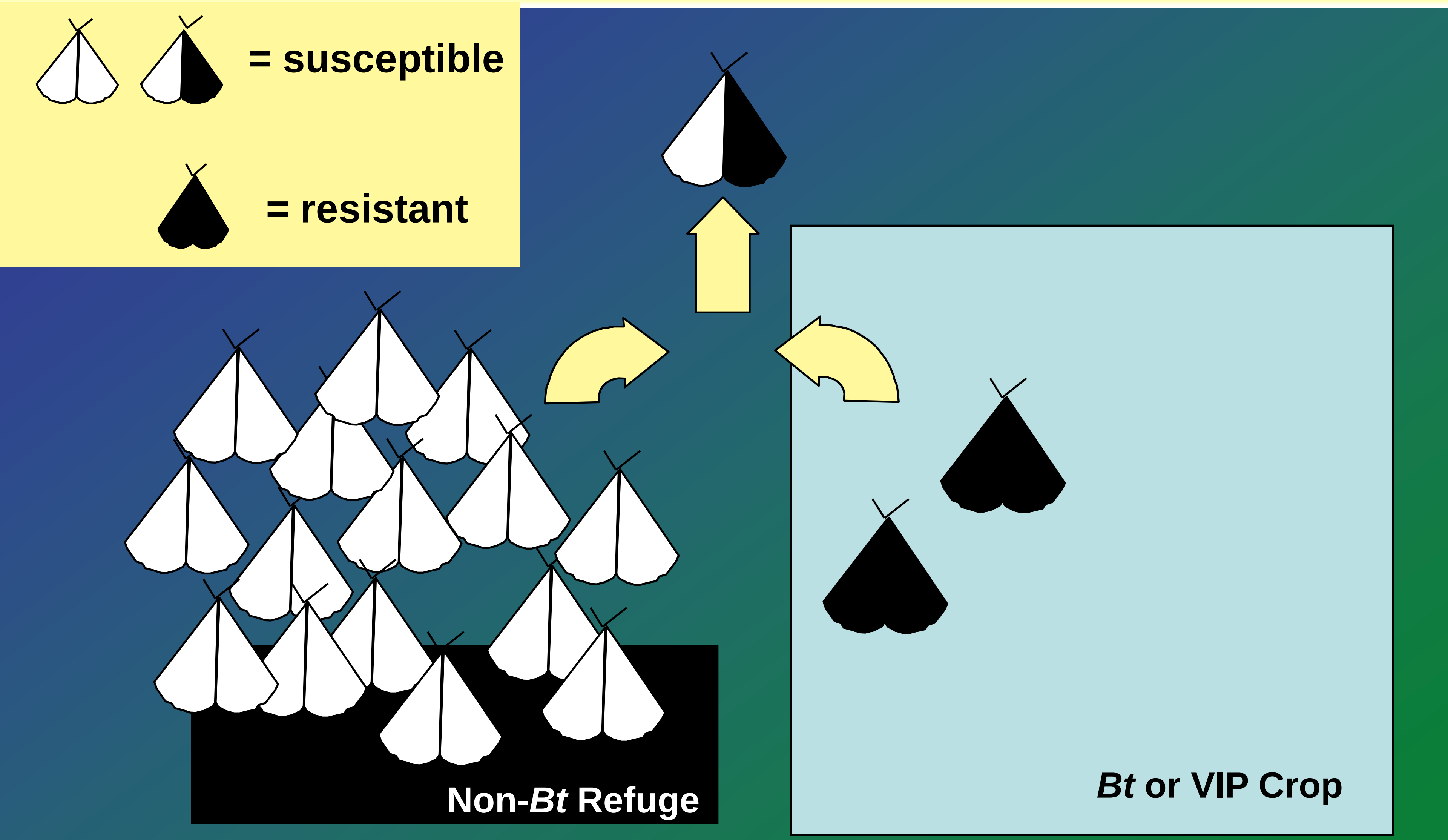
Experience with IRM Tactics

- Initial IRM plans were based on:
  - Crops expressing a 'high dose' of the Cry or VIP proteins
    - High enough to kill both susceptible insects and heterozygous offspring when resistance trait is recessive
  - A structured refuge, planted close to or within the insecticidal crop, to produce large numbers of susceptible insects
  - Monitoring of the insecticidal crop to look for higher than expected levels of damage that might indicate development of resistant insect populations
  - The assumption that resistance would be functionally recessive and rare in insect populations

In theory…

Insect resistance will be delayed,
when resistant alleles are rare and
when the refuge produces sufficient numbers of susceptible insects to vastly outnumber the rare resistant insects that might emerge from the insecticidal crop.

Refuge Strategy: Recessive Trait



IRM Logo for *Bt* Crops in USA

Insect
Resistance
Management

Planting Refuges, Preserving Technology

An Example: *Bt* Maize in the USA

<table>
<tr><th>Trait target</th><th>Refuge size</th><th>Deployment</th><th>Proximity</th></tr>
<tr><td>Corn borer</td><td>20% (corn regions)
50% (cotton regions)</td><td>Discrete CB refuge</td><td>Internal or external blocks within ½ mile (¼ mile preferred) or in-field strips (at least 4 rows wide)</td></tr>
<tr><td>Root worm</td><td>20%</td><td>Discrete RW refuge</td><td>Internal or external blocks adjacent or in-field strips (at least 4 rows wide)</td></tr>
<tr><td rowspan="2">Corn borer + Root worm</td><td rowspan="2">20% (corn regions)
50% (cotton regions)</td><td><u>2 options:</u>
1. Common RW/CB refuge</td><td>Internal or external blocks adjacent or in-field strips (at least 4 rows wide)</td></tr>
<tr><td>2. Discrete RW and CB refuges</td><td>Separate fields should be used within ½ mile (¼ mile preferred)</td></tr>
</table>

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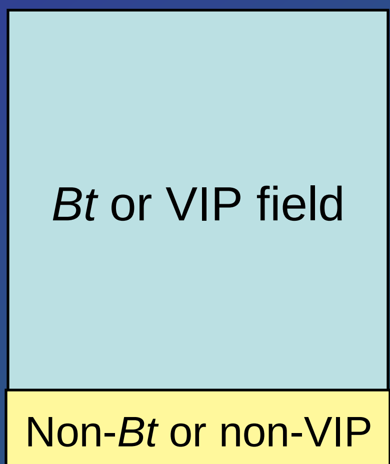
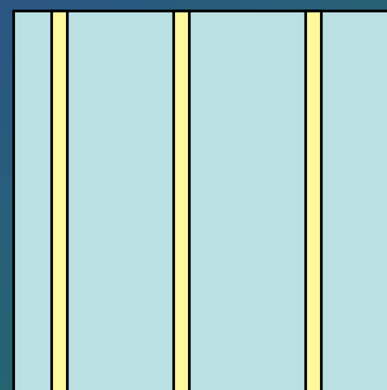
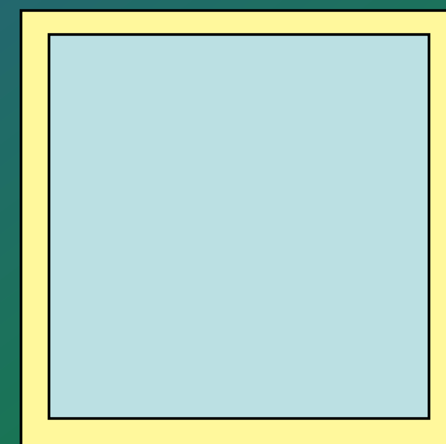
Refuge Deployment Options: Maize



Block



Strips



Border

Pyramided Bt Crops & Improved IRM Plans

- Optimized IRM plans have been developed based on experience and research results
- Insecticidal crops that express 2 or more insecticidal proteins are now commercial: called pyramided crops
  - The Cry and/or VIP proteins control the same insect
  - Killing the insect by unique sites of action (binding sites)
  - Pyramided insecticidal crops have a lower risk of resistance
- Natural refuge can be an important source of insects, especially generalists – insects that feed on a wide variety of crop species
  - Natural refuge consists of all types of different non-crop plants that can serve as hosts for the target pest

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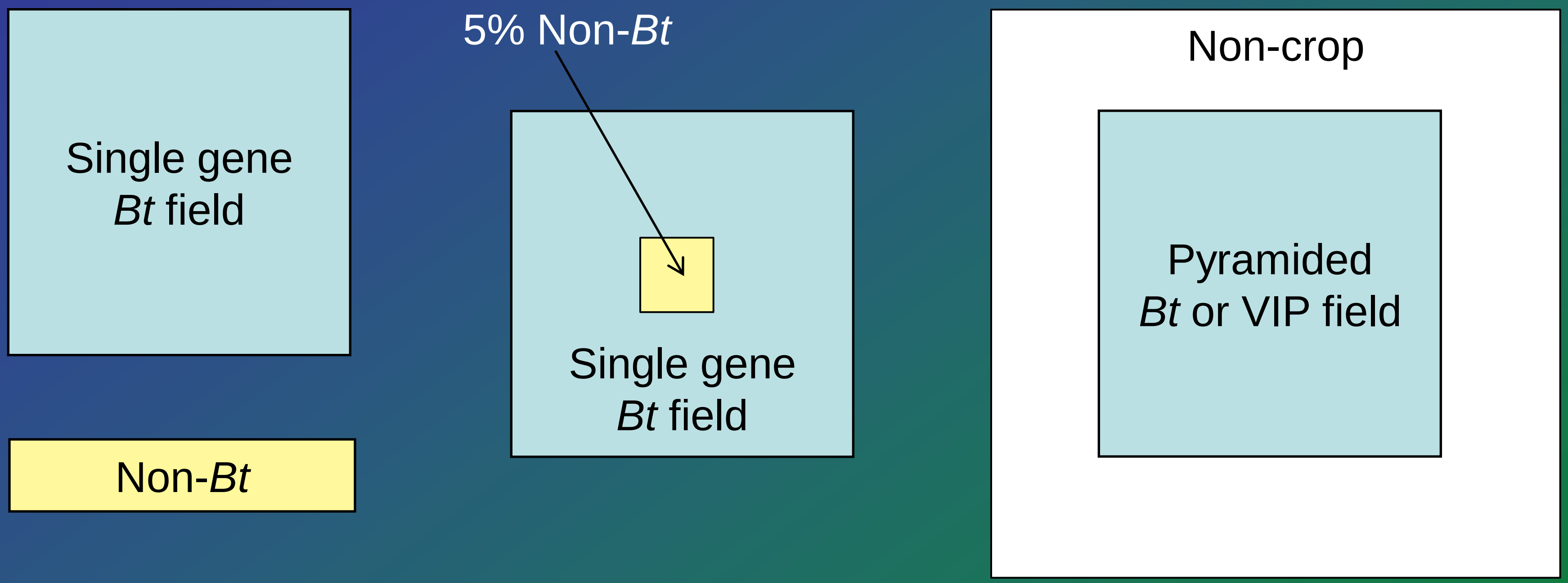
An Example: *Bt* Cotton in USA

Gene #	Region	Refuge size	Deployment	Proximity
Single	All	1. 5% external unsprayed 2. 5% embedded 3. 20% external sprayed[2]	1. At least 50m wide 2. At least 50m wide 3. N/A	1. ½ mile (¼ mile preferred) 2. Embedded in field 3. 1 mile (½ mile preferred)
Dual	AZ, CA, NM, west TX			
Dual	Southeast US	Natural refuge – no structured refuge requirement		
Single / dual	For PBW only – AZ and CA	N/A	At least one row for every 6 to 10 rows of Bt cotton	Embedded in field

Refuge Deployment Options: Cotton



20% External Block

Embedded

Natural Refuge

An Example: Pyramided Maize Products

- Pyramided maize products, MON89034 and Bt11xMIR162, have been granted reduced refuge in the US
- In cotton growing regions, both products have been granted a 20% non-Bt corn refuge (reduced from 50%)
  - Both products offer two modes of action vs *H. zea*
- Only MON89034 has been granted a reduced refuge in corn growing regions to 5% (reduced from 20%)
  - Bt11xMIR62 only offers one mode of action vs. *O. nubilalis*
- Deployment and Proximity requirements remain unchanged from single gene maize products

Other IRM Plan Elements – USA

- Monitoring for susceptibility of the target pest(s)
  - Collect insect populations from greatest concentration of insecticidal crop areas or in association with crop damage
  - Routinely conduct insect bioassays using specialized screening techniques
  - Goal: Identify population shifts in sensitivity to the Bt and/or VIP active that indicate increasing risk of resistance occurring and need for proactive remedial action

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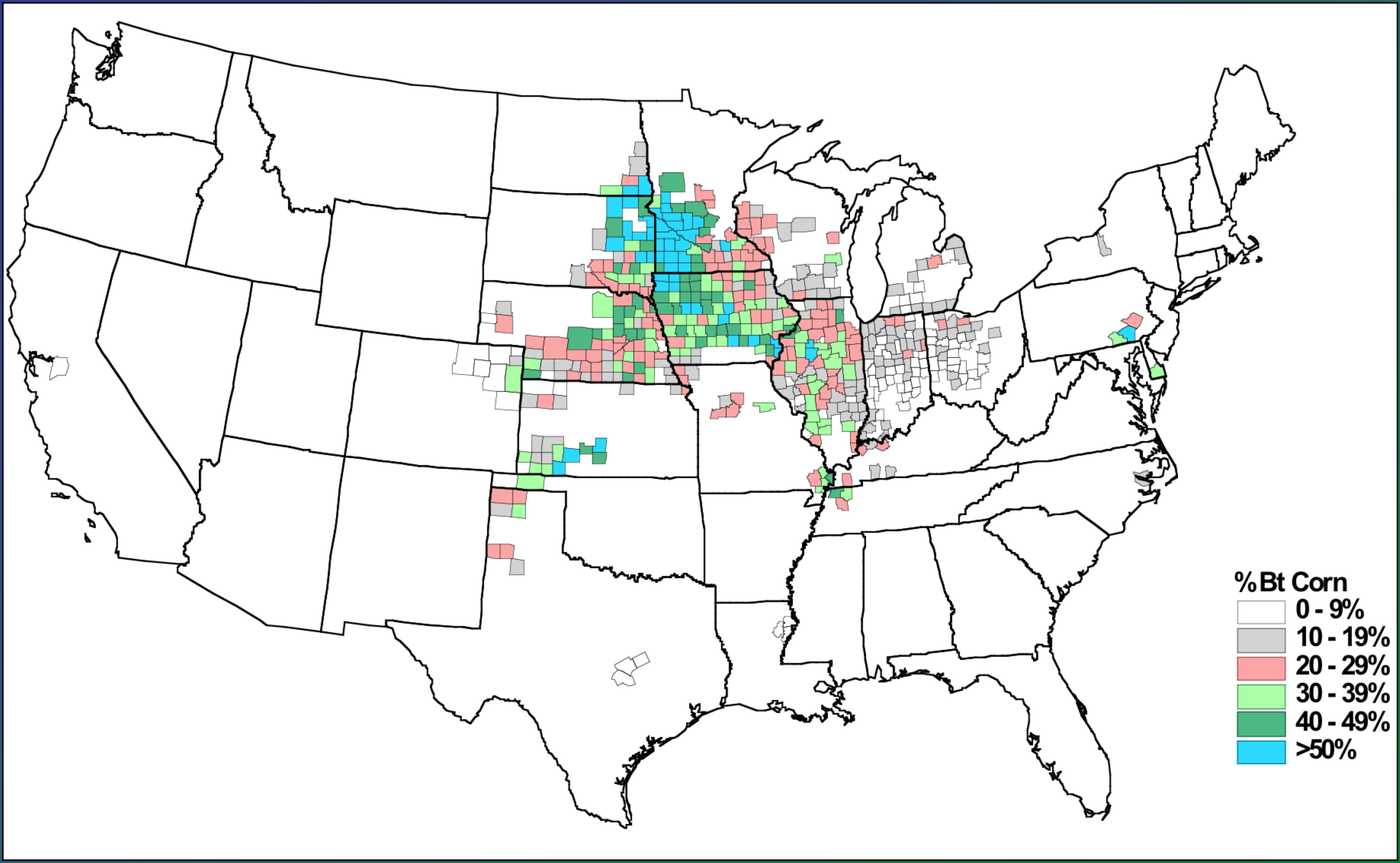
Bt Maize Growing Regions – USA



Resistance Monitoring Regions - Central Corn Belt

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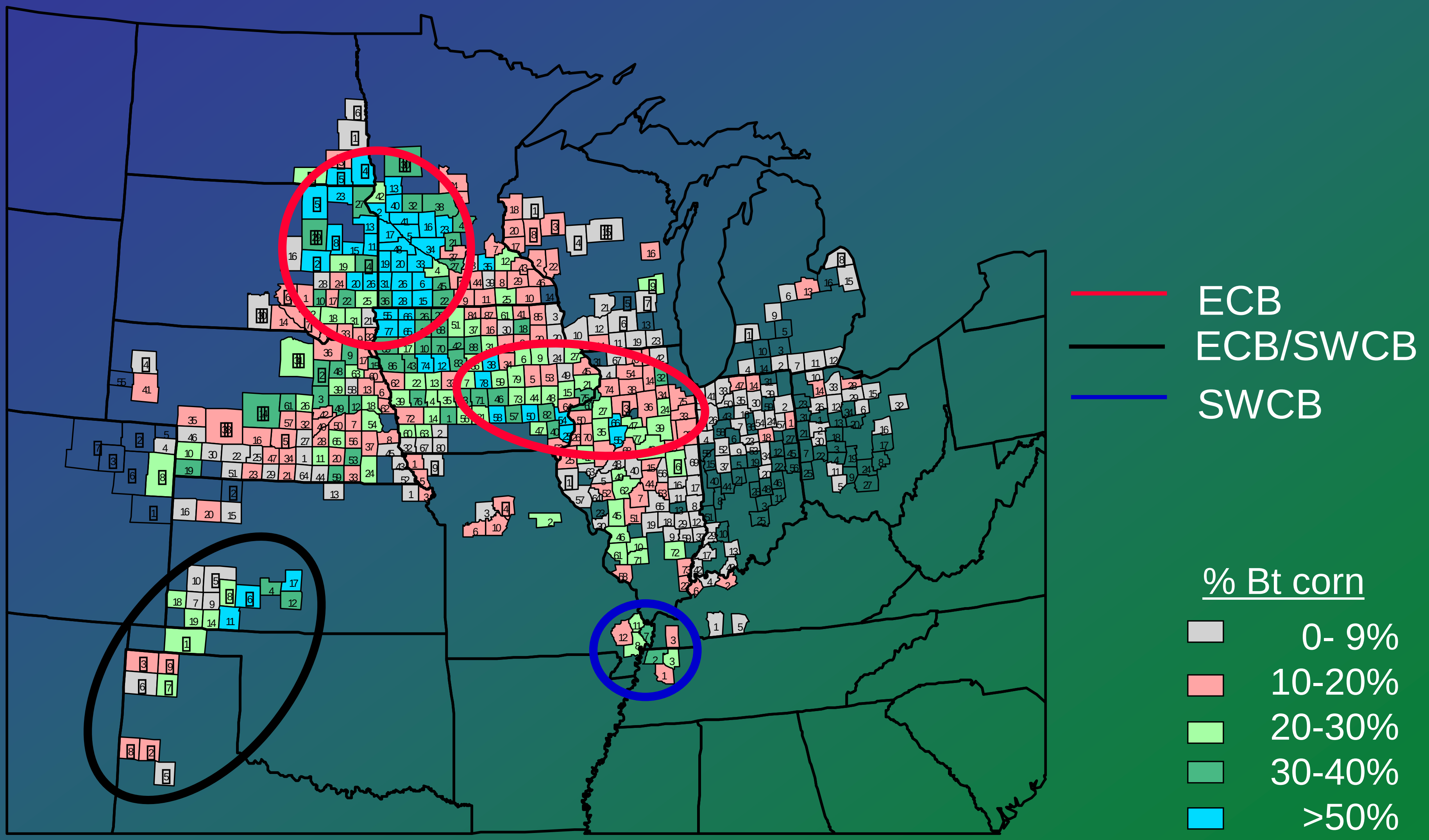

Other IRM Plan Elements – USA

- Education and communication for growers and other stakeholders
  - Appreciation for proper implementation of IRM plans
- Monitoring for grower compliance of IRM plans
  - Is refuge deployed according to the plan?
  - Re-enforcing education, when growers are found out of compliance
- Remedial action plans when resistance is suspected and/or confirmed

Experience vs. Local Needs

While experience is important, the local environment is equally as important!

THEREFORE,

what has worked for large plantings of monoculture production systems in North America may not be effective for small, diverse agricultural farms found in other regions

IRM Plans: Australia

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- Initial IRM plans limited each grower to planting a maximum of 30% of his farm to Bt cotton
  - Specifically recognized the higher risk of Bt crops with a single Cry protein
- Planting times are restricted
- Limited insecticide use on refuges
- Require post-harvest cultivation to reduce overwintering populations
  - 'Pupae busting'
- Once pyramided Bt cotton was commercialized; the planting cap of 30% was lifted

IRM Plans: China

- No structured refuge requirement
  - Growers farm plots of 5Ha or less
  - Mixtures of crop species are present
  - Thus abundant natural refuge is available
- Annual monitoring for insect susceptibility is conducted for all commonly used insecticides, including the Cry proteins in Bt cotton
  - Old-World Cotton Bollworm is primary target pest
- To date cotton (Bt and Bt/CpTI) is the only Bt crop registered for cultivation, limiting insect selection pressure

IRM Plans: India

- Growers are requested to plant a non-Bt refuge around each plot of Bt cotton
  - Refuge must be at least 5 rows wide
  - The non-Bt refuge must be at least 20% of the total cotton plot
- The company supplies the non-Bt seed in a package attached to the Bt cotton seed
  - For each 450g Bt cotton seed, 120g non-Bt cotton seed is included
- Frequent monitoring of insect susceptibility is conducted

How to Develop an IRM Plan

Several elements are common to all IRM plans:

- Identify key stakeholders to participate in IRM plan development and maintenance
  - Scientific experts on crop and pest biology
  - Grower groups/advisors know the local production practices
  - Commodity groups and product developers can provide education and communication about the technology
  - Regulatory authorities can ensure compliance with IRM plans

How to Develop an IRM Plan

- Information about crop biology, target pest biology and crop/pest interactions should be determined
  - Insect movement and feeding patterns are key
  - Does the *Bt* crop provide a high level of insect control?
  - Pyramided Cry and/or VIP proteins?
- Local growing conditions should be described
  - Are crops grown in large monocultures?
  - Or are mixtures of crops more prevalent?
  - How big is the typical farm size?
  - How will local or regional cultural practices impact IRM plans?

How to Develop an IRM Plan

- Growers and all stakeholders should fully appreciate the insecticidal crop technology, the superior safety profile and how it is best deployed for long term durability
  - What pests will be controlled?
  - What are IRM plan tactics and requirements? Will there be…
    - Refuges deployed?
    - Limits on insecticidal crop sales?
    - Monitoring market penetration to set action triggers?
    - Cultural practices to limit insect selection pressure?
  - What are all the crop deployment options?

How to Develop an IRM Plan

- The responsibilities should especially be clear to the grower through education and communication
  - Is there a maximum amount of insecticidal crop that can be planted at one farm?
  - Is a non-insecticidal refuge required?
    - ✓ If so, how much? And where should the refuge be placed?
  - Can insecticides be used?
    - ✓ On the insecticidal crop? On the non-insecticidal refuge? Both?
    - ✓ If so, what is the economic threshold?
  - How does the grower report unexpected insect damage?
  - What records should be kept?

How to Develop an IRM Plan

- Establish insect susceptibility baseline
- Establish routine monitoring plan for insect susceptibility and for grower compliance
  - Decide the frequency for all monitoring
- Monitoring for insect susceptibility
  - Develop a method for screening insect populations
    - ✓ Discriminating dose assay is one method
  - Collect representative insect populations from areas where the highest amount of insecticidal crop is planted
  - Conduct routine insect and field monitoring plan

How to Develop an IRM Plan

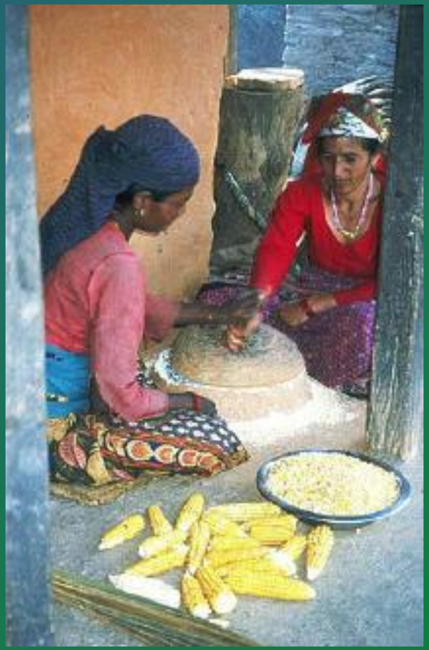
- Monitoring for grower compliance to IRM plan
  - Determine an unbiased method to assess compliance
  - When growers are found out of compliance, what are the consequences?
    - ✓ Additional education or incentives?
    - ✓ Remove access to insecticidal crop technology?
- Remedial action plans established before or soon after commercial launch

Thank you for your attention!

Are there any questions?

Bt Maize Reduced Fungal Toxins

- Fungi can produce harmful toxins in our crops
- Mycotoxins cause acute illness and cancer in humans and animals
  - Insect damage on crops allows fungi to grow that produces mycotoxins
  - Because Bt maize reduces insect damage, fungal growth is also reduced
- *Bt* maize reduces mycotoxin levels to ½ of conventional maize
- Beneficial for animal and human health



Mycotoxins in Maize

Fumonisin	*Fusarium verticillioides, F. proliferatum*
Aflotoxin	*Aspergillus flavus, A. parasiticus*
Deoxynivalenol (DON, vomitoxin)	*F. graminearum, F. culmorum*
Zearalenone	*F. graminearum, F. culmorum*

Examples of Ear Rot

Fusarium *Gibberella* *Aspergillus*

Photos: Gary Munkvold

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Results: *Bt* Maize & Fumonisin