WideStrike (Cry1F + Cry1Ac) Cotton: Issues Related to Establishing an IRM Plan

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Purpose

- Present various aspects of EPA's analysis of Dow's WideStrike cotton IRM submissions
  - Dose studies for *Heliothis virescens* (TBW, tobacco budworm), *Helicoverpa zea* (CBW, cotton bollworm), and *Pectinophora gossypiella* (PBW, pink bollworm)
  - Pest adaptation likelihood including modeling and IRM Plan
- EPA will decide on the acceptability of Dow's WideStrike IRM Plan.

Outline

- Key IRM issues
- What is WideStrike cotton
- Target pests
- Factors in pest adaptation
  - Operational factors
  - Biological factors
  - Genetic factors

- Models
- Resistance management plan
- SAP Questions

Outline

- **Key IRM issues**
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Key IRM Issues for SAP

- Dose

- Cross-resistance potential (Cry1F and Cry1Ac)

- Cotton bollworm alternate hosts

- Models – tobacco budworm and cotton bollworm

- IRM Plan

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What is WideStrike Cotton?

- Event 281-24-236 (Cry1F)) X Event 3006-210-23 (Cry1Ac)

    = Event 281-24-236/3006-210-23

- WideStrike expresses both Cry1Ac and Cry1F
- Cry1Ac expressed in Bollgard^{TM} cotton and Bollgard^{TM} II - Cry1Ac + Cry2Ab
- Cry1F expressed in Herculex^{TM} corn

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Key Lepidopteran Pests of Cotton

- **Major pests:**
  - tobacco budworm (*Heliothis virescens*, TBW)
  - cotton bollworm (*Helicoverpa zea*, CBW)
  - pink bollworm (*Pectinophora gossypiella*, PBW)
- **Secondary pests:**
  - cabbage looper (*Trichoplusia ni*, CL)
  - soybean looper (*Pseudoplusia includens*, SL)
  - beet armyworm (*Spodoptera exigua*, BAW)
  - fall armyworm (*Spodoptera frugiperda*, FAW)
  - southern armyworm (*Spodoptera eridania*, SAW)

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Factors in Pest Adaptation

- Operational factors
- Biological factors
- Genetic factors
- IRM Plan must consider all factors

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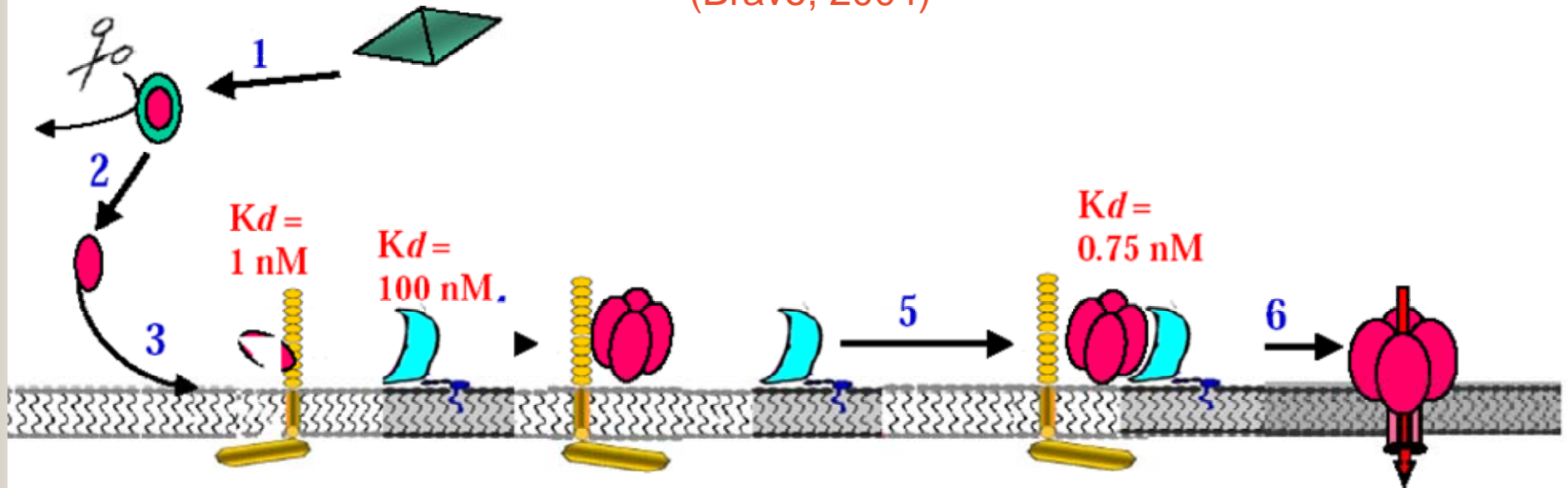- SAP Questions

Operational Factors

- Mode of action
- Target
  - Larval effects
  - No adult effects – no insecticidal crystal proteins (ICPs) expressed in nectar
- Dose and functional dominance
- Pyramided Toxins
  - Cry1F + Cry1Ac
- Market share

Mode of action of Cry1A toxins

(Bravo, 2004)

$Kd = 1\ nM$

$Kd = 100\ nM.$

$Kd = 0.75\ nM$

1.- Crystal solubilization
2.- Protoxin activation
3.- Monomer binding to cadherin
    and cleavage of helix α-1
4.- Oligomer fomation

5.- Oligomer binds APN
6.- Pore formation in lipid rafts.

Pore
Formation

Other events?

Cry1Ac and Cry1F Expression

Tissue	Cry1F Mean Expression (ng ICP/mg tissue)	Cry1Ac Mean Expression (ng ICP/mg tissue)
Young leaves	6.81	1.82
Terminal leaves	8.19	1.31
Squares	4.88	1.82
Bolls	3.52	0.64
Flowers	5.44	1.83
Pollen	0.06 (<LOQ)	1.45
Roots (defoliated, pollination, seedling)	0.51, 0.54, 0.88	N.D., <LOQ, 0.17
Nectar, meal, oil	Not Detectable (N.D.)	N.D.

High Dose/Refuge Assumptions

- Single or major resistance gene
- Recessive inheritance
- RRs are rare
- Refuge supplies SS (unselected source)
- Random mating or preferential mating – RR with SS
- High dose (1998 and 2000 SAP)
  - 25X the dose required to kill 99% of the susceptible insects
  - >95% RS will be killed and >99.9% of the susceptible insects will be killed

Methods to demonstrate high dose

1. Serial dilution bioassay with artificial diet containing lyophilized tissues of Bt plants using tissues from non-Bt plants as controls. **(TBW and PBW)**

2. Bioassays using plant lines with expression levels approximately 25-fold lower than the commercial cultivar.

3. Survey large numbers of commercial plants in the field to see if Bt plants are at the LD99.9 or higher to assure that 95% of the heterozygotes will be killed. **(CBW – NC)**

Methods to demonstrate high dose (cont.)

4. Similar to #3, but would use controlled infestations with a laboratory strain of the pest that had an LD50 value similar to field strains. (**CBW – MS; PBW; TBW**)

5. Determine if a later instar with an LD50 that was 25X higher than that of the neonate could be tested on Bt plants to determine if 95% or more of the later stage larvae were killed. (**TBW**)

Dose and Functional Dominance

- **TBW**
    - High dose for Cry1Ac + Cry1F(>99.9%)
    - High dose for Cry1Ac alone
    - Nearly high dose for Cry1F alone
- **PBW**
    - High dose for Cry1Ac (>99.9%), Cry1F non-toxic
- Resistance likely to be functionally recessive, RS likely to be killed on WideStrike

- **CBW**
    - Not a high dose for Cry1Ac + Cry1F
    (~ 94% mortality – MS + NC)
    - Not a high dose for either ICP alone, but Cry1Ac >Cry1F
    - Resistance less likely to be functionally recessive
- **SAP Question #1: dose and functional dominance**

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Biological Factors

- Adult movement and dispersal

- Larval movement

- Alternate hosts

- Population dynamics

- Metapopulation dynamics - important consideration for CBW

Adult and Larval Movement

Pest	Adult	Larvae
TBW	Moderate (Fitt,1989)	Mobile
PBW	Limited (Tabashnik et al., 1999)	Not Mobile
CBW	Extensive (Fitt, 1989)	Mobile*

*In absence of a high dose, the consequence of larval movement on the population rate of adaptation is relatively small, since heterozygote survival is already relatively high compared to SS larvae.

Alternate Hosts

- HOSTS database for Nearctic region (host plants of the world's Lepidoptera)
- **TBW:**
  - 66 species from 20 families
  - wild hosts early and late
  - ability of alternate hosts to support complete insect development during the summer is unclear
- **PBW:**
  - 26 species from 5 families
  - most closely related to cotton
  - non-cotton hosts not important to adaptation

Alternate Hosts

- **CBW:**
  - 108 species from 30 families
  - Long-distance dispersal then host plants outside the immediate cotton-growing area act as important sources of non-selected populations [Metapopulation dynamics]
  - Gould et al. (2002) - carbon-isotope ratios in CBW adults collected in the mid-south and southwest US, indicate the more insects emerge from alternate hosts than from cotton for most of the year

Alternate Hosts

- Host data base – extensive alternate hosts
- CBW Model
    - Includes alternate hosts in two agroecosystems (NC and Delta)
    - Includes multiple ICPs (Cry1F, Cry1Ac, Cry2A)
- Gould et al. 2002 carbon isotope analysis showing N-S movement of CBW and influence of alternate hosts
- SAP Question #3: scientific adequacy of database and whether additional field data are needed

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Genetic Factors: Functional Dominance

- Functional dominance of R-allele (Is the R-allele genetically completely recessive)
  - TBW and PBW, Dominance = <0.05, functionally recessive [95% heterozygotes killed]
  - CBW, Dominance = 0.5, additive

Genetic Factors: Initial R Frequency

- Initial R frequency is rare
  - TBW: 0.0015, recessive (Gould et al. 1997)
  - CBW: 0.00043, incompletely dominant (Burd et al. 2001)
  - PBW: variable, 0 to 0.16 (Tabashnik et al. 2000); R-allele frequency dropped in subsequent years, recessive; fitness costs to resistance - overwintering and survival on non-Bt cotton plants (Carrière et al. 2001b and c)

Genetic Factors: Cross-Resistance

- Cross-resistance among *Bt* ICPS
    - Potential for genes that confer cross-resistance by reduced binding
    - Potential to occur through protein digestion in the midgut, broad cross-resistance
    - SAP Question #2: cross-resistance potential Cry1F and Cry1Ac
- Cross-resistance between Bt and other control mechanisms (e.g., pyrethroids, carbamates, spinosad)
   - Not expected based on mode of action

Cross-Resistance Potential: Cry1F and Cry1Ac

- Resistance to *Bt* proteins may occur through several different mechanisms.
- Two common *Bt* resistance mechanisms (Ferré and Van Rie, 2002; Tabashnik, 1994):
  - Detoxification
  - Receptor binding modification (most common)
- Loss of function (i.e., binding), resistance expected to be recessive (Ferré and Van Rie, 2002)
- Cry1F and Cry1Ac – share binding sites and have unique binding sites in TBW and CBW
- Shared binding may lead to cross-resistance, enhance survival against both Cry1Ac and Cry1F

Cry1Ac and Cry1F Binding Patterns in TBW and CBW

- Protein-pest specificity is mediated by ICP-binding midgut receptors
- Cry1F and Cry1Ac: shared and unique binding sites
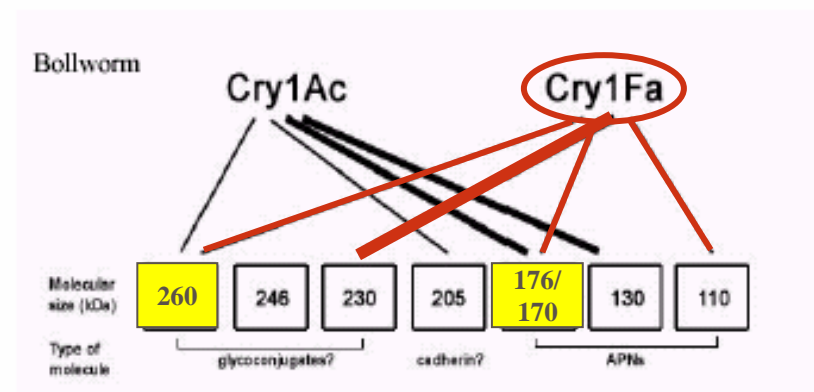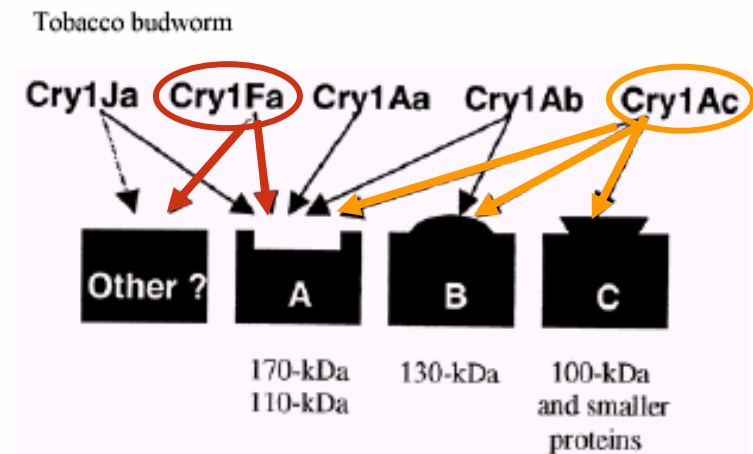
Binding Map for Cry1 proteins in TBW (top graphic) and CBW (bottom graphic). From Jurat-Fuentes and Adang (2001) and Adang et al. (2002) respectively.

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Cross-Resistance Potential - CBW

- Radiolabeled Cry1Ac (Dow: Sheets and Storer, 2001):
  - 60% of Cry1Ac binding is to Cry1F receptors, 40% of Cry1Ac binding is to non-Cry1F receptors
  - Incomplete shared receptor binding = incomplete cross-resistance when resistance is mediated by receptor changes.
  - Thus, a mutation in a gene that codes for a receptor that binds both Cry1Ac and Cry1F will not prevent all binding to either ICP, and thus alone, will not allow high survival of the insect bearing even two copies of it on WideStrike cotton.
- Radiolabeled Cry1F - Problems with radiolabeling affect the Cry1F activity, but expect incomplete shared receptor binding and same conclusions as above

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Models

- Peck et al. (1999) for TBW
- Dow (2003) for CBW
- SAP Questions #4 and #5 regarding TBW and CBW models

Peck et al. (1999) TBW Model

- Spatially explicit, stochastic model
- Examined refuge size and spatial pattern of *Bt* and non-*Bt* plants (such as seed mixes and external refuge) on resistance development, and the effects of varying the spatial pattern each year.
- 20% refuge delayed resistance.
- Greater durability if refuge remains the same from year to year, but localized resistance foci
- If structured refuge is moved each year, resistance predicted in approximately 17 years.

Peck et al. (1999) TBW Model

- Durability for WideStrike greater than predicted by Peck et al. (1999) - >>10X
  - Simulations of pyramided ICPS (e.g. Roush 1997, Gould 1998) show that adding an additional ICP to the plant always delays the development of resistance to each ICP individually
  - Initial R-allele frequency of 0.03, value much higher than the frequency of Cry1Ac R-alleles in TBW populations, 0.0015 (Gould et al. 1997)

Dow CBW Model

- Adapted Storer et al. (2003) CBW model for WideStrike
- Spatially-explicit, stochastic
- Alternate hosts
- North Carolina and Mississippi Delta agroecosystems
- Multiple ICPs: Cry1F, Cry1Ac, Cry2Ab
- 15-year time horizon

DAS CBW Model cont…

- R-allele frequency (unmutated, no fitness costs) = 0.001
  - 1 in 1,000,000 individuals will be homozygous for the mutated form of one of the receptors.
  - 4 in 1,000,000 will be heterozygous for the mutated form of two receptors.
- R-alleles are assumed to be functionally additive, moderate dose (i.e., functional dominance = 0.5)
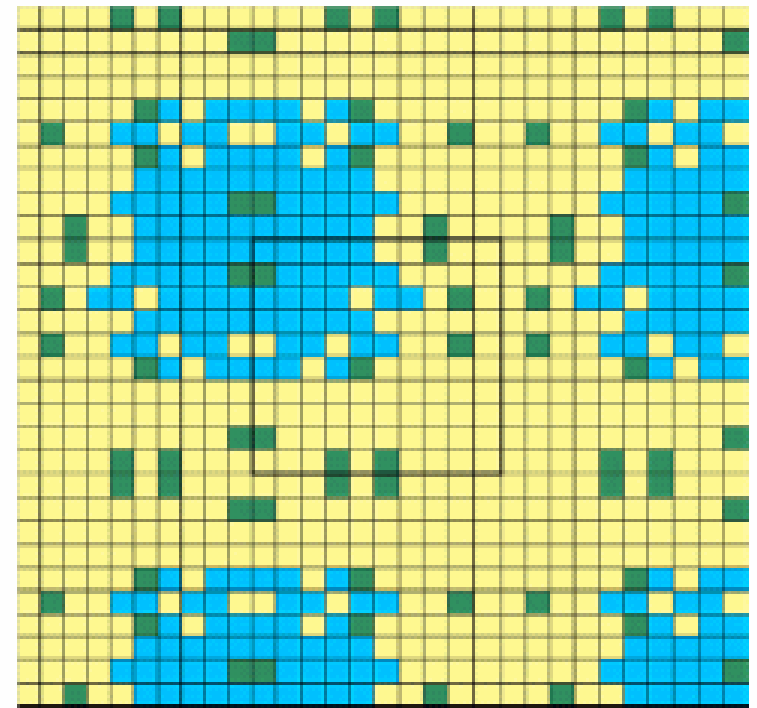
Agroecosystems in Model

A **North Carolina**
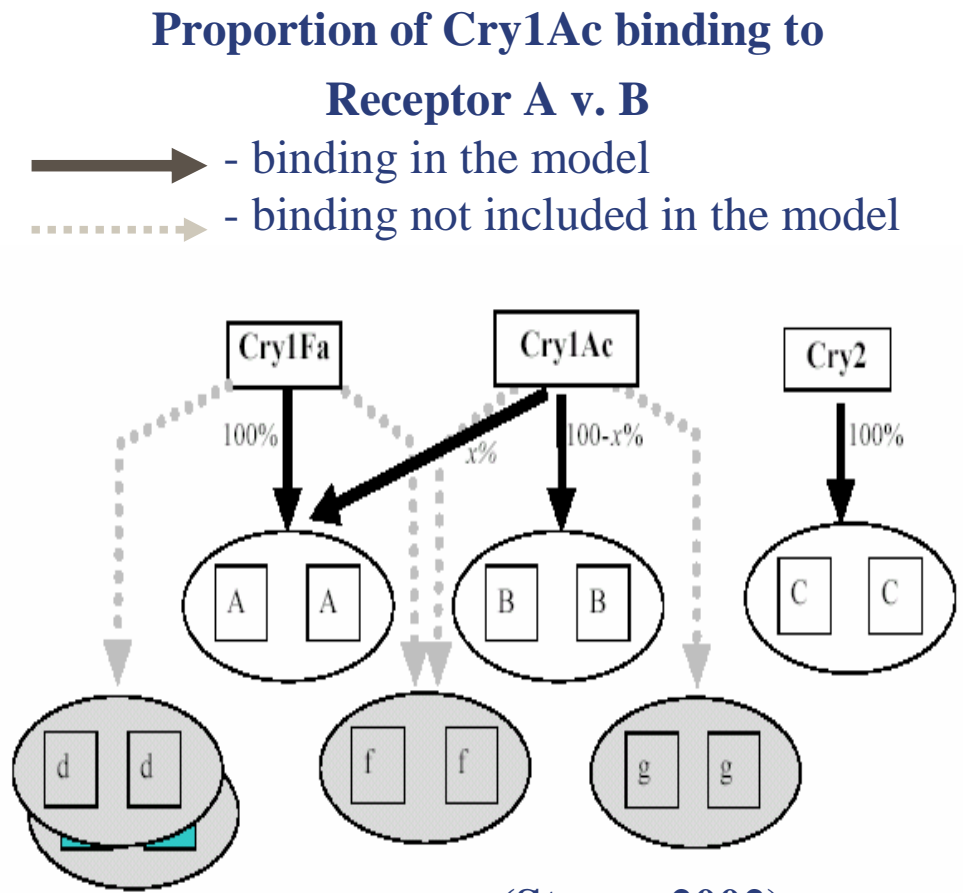50% soybean, 25% maize, 25% cotton
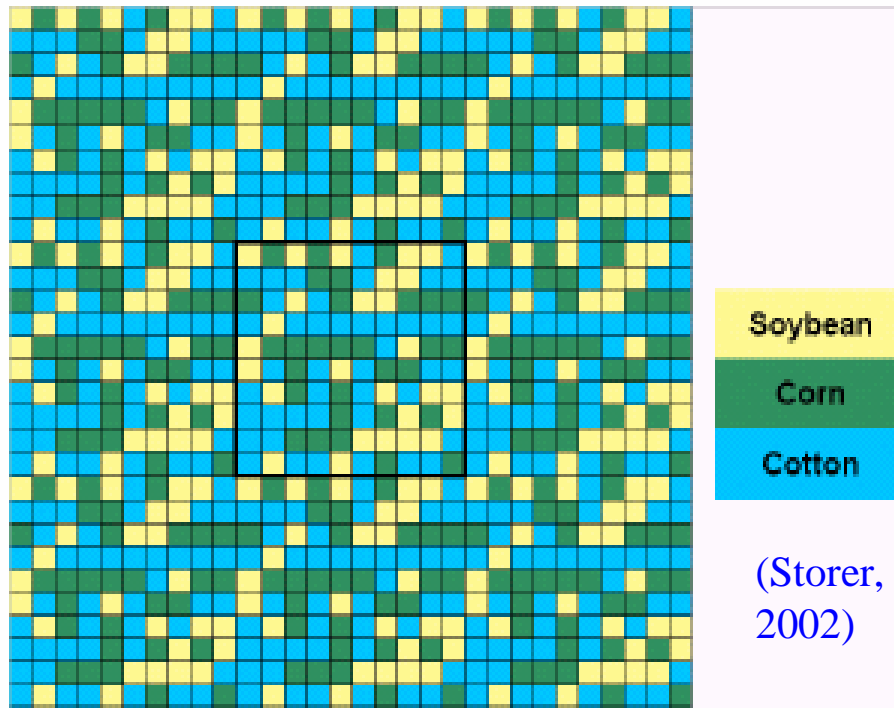
B **Mississippi Delta**
62% soybean, 8% maize, 30% cotton

Soybean

Corn

Cotton

(Storer, 2002)

Maize- 1st two generations; cotton - 2nd two generations each year; weeds – 1st and last generations; soybeans – 2nd and 3rd; 10 X 10 fields modeled

DAS CBW Model: Simplified Binding Map

- Simplified possible receptors to 3 (A, B, and C) from 6 or more

- There are two loci at which R-alleles can lead to adaptation to WideStrike; one for receptor A and one for receptor B.
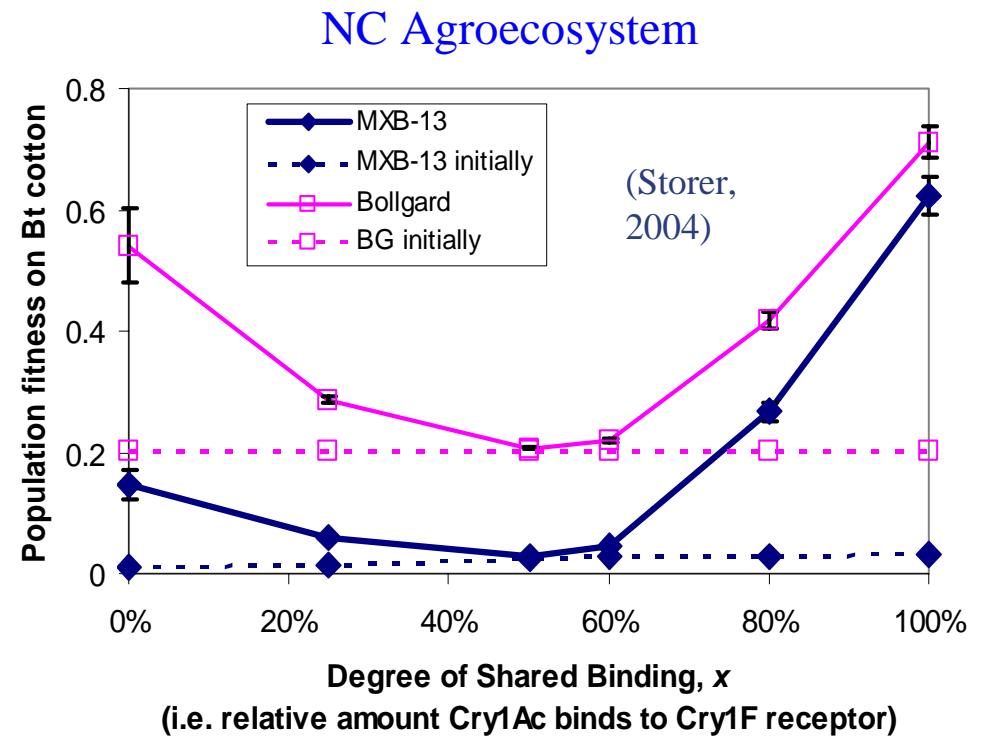
Proportion of Cry1Ac binding to Receptor A v. B

- binding in the model
- binding not included in the model

Cry1Fa   Cry1Ac   Cry2

100% $x\%$ $100-x\%$ 100%

A A   B B   C C

d d   f f   g g

(Storer, 2002)

Impact of Shared Binding on Population Fitness – Sensitivity Analysis

- *x = 20-60%: Intermediate level cross-resistance*
  - Selection at both loci occurs on all *Bt* cotton and *Bt* corn, least change in population fitness.
  - Adaptation to Cry1Ac and Cry1F occurs most slowly.
  - Selection pressure exerted by Cry1Ac>Cry1F because Cry1Ac in Bollgard, Bollgard II, and WideStrike
  - Binding data indicate that intermediate levels are appropriate: 60% of Cry1Ac binds to Cry1F receptor
- *x = 0%: No cross-resistance*
- *x = 100%: Complete cross-resistance*

NC Agroecosystem

(Storer, 2004)

Legend: MXB-13; MXB-13 initially; Bollgard; BG initially

Y-axis: Population fitness on Bt cotton (0 to 0.8)
X-axis: Degree of Shared Binding, *x* (i.e. relative amount Cry1Ac binds to Cry1F receptor) (0% to 100%)

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CBW Mortality for Model

- Cry1F line alone: 67%
- Cry1Ac line alone: 97%
- WideStrike (Cry1Ac + Cry1F): 97.2%, Cry1Ac shared binding = 60%
- Bollgard (Cry1Ac alone): 80% (Lambert et al. 1997)
- Bollgard II (Cry2Ab2 + Cry1Ac): 96%

CBW Fitness Values for Model

- To understand mortality of insects carrying one or more R-alleles to understand the durability of WideStrike
  - Functional dominance of resistance on each *Bt* cotton type
  - Degree of shared binding: 60% of Cry1Ac binds to Receptor A, 40% to Receptor B
- R-alleles are assumed to be functionally additive, functional dominance = 0.5, due to lack of high dose

- Two loci at which R-alleles lead to adaptation to WideStrike (A and B)
  - Cry1Ac fitness depends on genotypes for Receptor A and B.
  - Cry1F fitness depends on genotype for Receptor A
  - Cry2 fitness depends on genotype for Receptor C
- Calculate fitness values for the model (27 possible genotypes)
  - Values indicate the survival probability of each genotype on each Bt cotton type.

CBW Adaptation Assumptions

- ICP binding to the individual binding receptors included in the model is all functional and leads to the insecticidal activity.

- Adaptation to the ICPs is assumed to be caused by mutations to the midgut receptors and that each receptor requires a different mutation

- Complete adaptation to both ICPs: insect would have to be homozygous for two receptor mutations

- Heterozygous insects (functional dominance = 0.5) have a fitness halfway between that of SS and RR insects

Market Share of WideStrike Vs. Bollgard or Bollgard II: Impact on Population Fitness
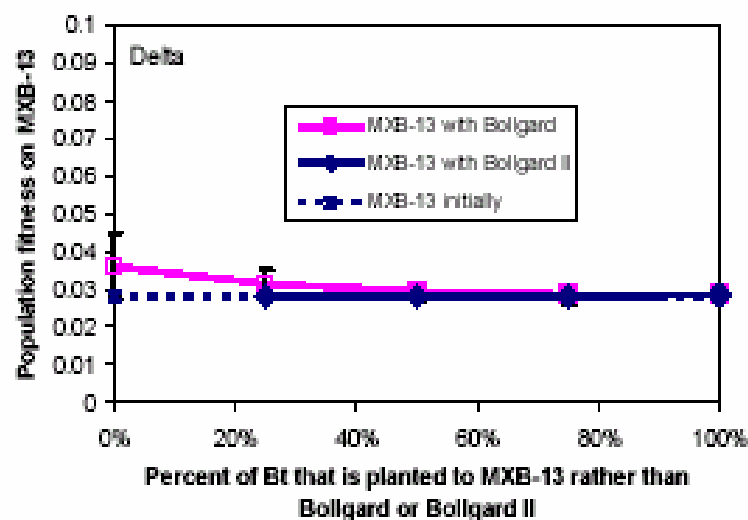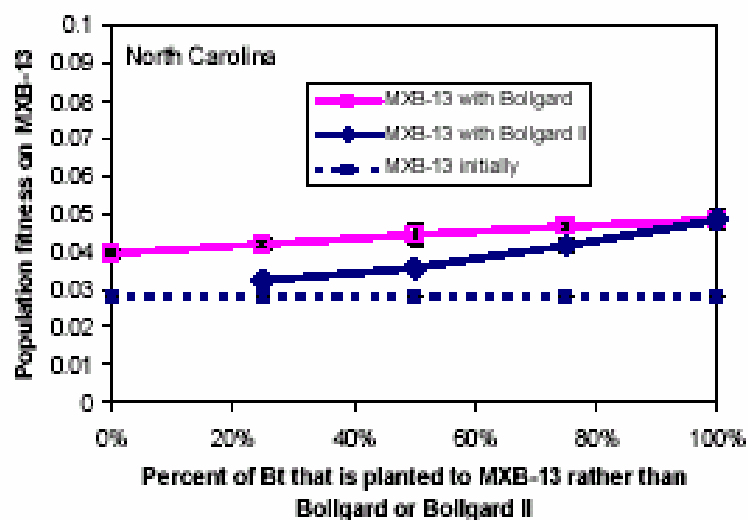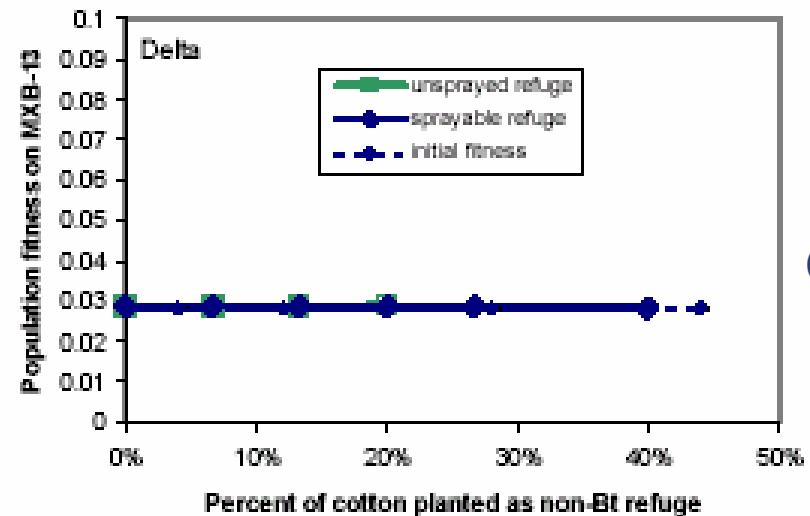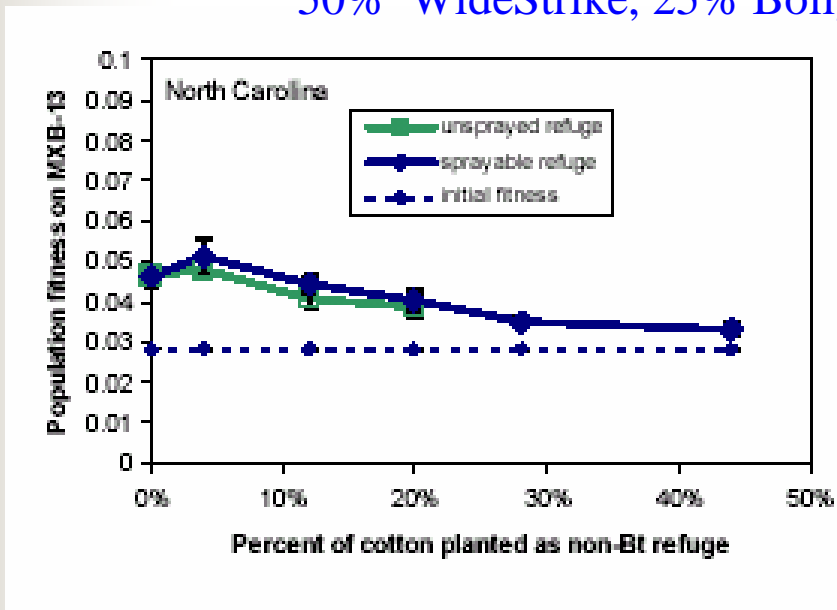

(Storer, 2002)

- Complex of ICPs reduces the selection pressure to any one product.
- WideStrike had minimal impact on the rate at which CBW may adapt in the Mississippi Delta and North Carolina agroecosystems.
- Marketshare of WideStrike with Bollgard II results in slower adaptation because insects are faced with 3 ICPs.
- In Delta, population fitness decreases due to influence of immigrants.

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Refuge Size Impact on Population Fitness

50% WideStrike, 25% Bollgard, 25% Bollgard II

(Storer, 2002

•Refuge size, whether sprayed or unsprayed, had minimal impact on CBW population fitness on WideStrike after 15 years.
•In the Delta, the immigrating non-selected population from alternate hosts further reduces the local rate of adaptation. The local structured refuge only supplies a small proportion of the non-selected insects.

Sensitivity Analysis

- Most important:
    - Proportion of the landscape planted to soybean
    - Soybean flowering dates
    - Immigration of non-selected populations
    - Initial R-allele frequency
    - Fitness costs of R-alleles.
- Moderate effects:
    - Functional dominance of R-alleles on each crop
    - Dispersal probability
    - Larval development duration.

WideStrike CBW Adaptation Conclusions

- WideStrike durability: Modeling indicates no significant change in population fitness in CBW over 15-year time horizon
  - Moderate dose for Cry1Ac and Cry1F against CBW
  - Incomplete cross-resistance – 60% shared binding
  - Modeling is conservative; fewer binding receptors
  - Alternate hosts add to durability; although more field data needed
  - Durability similar to Bollgard II (Cry1Ac + Cry2Ab)

WideStrike TBW Adaptation Conclusions

- WideStrike durability greater than predicted by Peck et al. (1999)
    - Widestrike stack (2 ICPs) expresses a high dose against TBW, more durable than high dose single gene product.
    - TBW exhibits similar patterns in binding studies as does CBW, i.e., some shared and some unique receptors.
    - Incomplete cross-resistance

WideStrike PBW Adaptation Conclusions

- WideStrike expresses a high dose of Cry1Ac, like Bollgard (Cry1Ac) cotton.
- Single gene product, Cry1Ac, for PBW
- Cry1F is not effective against PBW.

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WideStrike IRM Plan: Refuge Options

- **5% external, unsprayed refuge**: Five percent of the cotton fields must be planted to non- Bt cotton and not be treated with any lepidopteran-control technology. The refuge must be at least 150 ft. wide (preferably 300 ft.) and within ½ mile (preferably adjacent or within 1/4 mile or closer) of the Bt cotton.

- **20% external, sprayed refuge**: Twenty percent of the cotton fields must be planted to non- Bt cotton and may be treated with lepidopteran-active insecticides (or other control technology) except for microbial Bt formulations. The refuge must be within 1 mile (preferably within ½ mile or closer) of the Bt cotton fields.

WideStrike IRM Plan: Refuge Options Cont.

- **5% embedded refuge for TBW and CBW**: Five percent of a cotton field (or fields) must be planted with non- Bt cotton as a block within a single field, at least 150 ft. wide (preferably 300 ft. wide) or single field blocks within a one mile squared field unit. The refuge may be treated with lepidopteran-active insecticides (or other control technology) only if the entire field or field unit is treated at the same time.

- **For PBW**: One single row of a non- Bt cotton variety must be planted for every 6 to 10 rows of Bt cotton. This can be treated with lepidopteran-active insecticides (or other control technology) only if the entire field is treated at the same time.

WideStrike IRM Plan: Refuge Options Cont.

- **Community refuge**: Farmers can combine neighboring fields within a one-mile squared field unit that act as a 20% sprayable refuge or the 5% unsprayed refuge. Participants in the community refuge option must have a community refuge coordinator and appropriate documentation is required.

WideStrike IRM Plan Cont.

- Grower agreements and annual affirmation
- Grower education program
- Grower compliance program
- Monitoring for insect resistance
- Remedial action plans
- Additional research – CBW resistant colonies

WideStrike IRM Plan Conclusions

- Conservative plan for WideStrike:
  - Pyramided toxins, Cry1Ac and Cry1F, for TBW, CBW
  - Incomplete cross-resistance
  - TBW and CBW models predict high durability for at least 15 years
  - TBW: high dose for WideStrike; high dose for Cry1Ac alone, nearly high dose for Cry1F
  - CBW: high moderate dose for WideStrike
  - PBW: high dose Cry1Ac

WideStrike IRM Plan Conclusions

- Mix of Bt toxins (Cry1Ac, Cry1F and Cry2Ab) in market place will reduce selection pressure for adaptation --- especially for Cry1Ac found in Bollgard, Bollgard II, and WideStrike

- Any plan that focuses on TBW, CBW, and PBW should be adequate, to maintain susceptibility in secondary pests, such as armyworms and loopers

- Consistency with existing plans, practical and implementable

Acknowledgements

- Alan Reynolds, M.S., OPP/BPPD
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WideStrike IRM Questions for the SAP

1. *Dose- TBW, CBW, PBW.* The Agency asks the SAP to comment on the Agency's analysis of dose for TBW, CBW, and PBW, the likelihood that resistance will be inherited as a recessive trait, and its impact on insect resistance management for WideStrike cotton.

WideStrike IRM Questions for the SAP

2. *Cross-resistance.*

    - The Agency asks the SAP to comment on EPA's conclusion that incomplete shared binding of Cry1Ac and Cry1F receptors, in TBW and CBW, is expected to lead to incomplete cross-resistance and thus the likelihood of enhanced survival on WideStrike cotton is expected to be small.

    - Please comment on EPA's conclusion that resistance is more likely to be associated with receptor binding modifications rather than other mechanisms of resistance such as detoxification in the midgut lumen by proteases that cleave the insecticidal control protein(s), metabolic adaptations, protease inhibition, gut recovery, and behavioral adaptations.

WideStrike IRM Questions for the SAP

3. *CBW Modeling*. The Agency asks the SAP to comment on the predictions made by the DAS CBW model, i.e., the likelihood that the population fitness of CBW on WideStrike cotton in a 15-year time horizon will remain unchanged, even without a high dose for either Cry1Ac or Cry1F and incomplete cross-resistance (60% of Cry1Ac binds to the Cry1F receptor).

4. *TBW Modeling*. The Agency asks the SAP to comment on the relative WideStrike cotton durability against TBW using the Peck et al. (1999) model.

WideStrike IRM Questions for the SAP

5. *Alternate hosts.* The Agency asks the SAP to comment on:

■   a) the sufficiency of the WideStrike cotton database to address the issue of CBW alternate hosts as natural refugia, and,

■   b) whether additional data are needed on the larval and adult production of CBW on each alternate host for each generation relative to cotton and WideStrike cotton and the spatial scale and source of moth production to confirm the effectiveness of CBW alternate hosts as natural refugia.

WideStrike IRM Questions for the SAP

6. *Refuge requirements.* The Agency asks the SAP to comment on the scientific data available to support the proposed IRM plan and whether that data support a delay in resistance of TBW, CBW, and PBW resistance to the Cry1F and Cry1Ac proteins expressed in WideStrike cotton for at least 15 years.