# Keeping pace with Bt corn: From stacked traits to blended refuges

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# A brief history of Bt corn

**Mid to late 90's:**

- **First Bt corn released, primarily targeting corn borer**
- **High dose toxin, high mortality of larvae**
- **No field resistance to this day, ECB now an afterthought in many areas**

- ***2003-08:*** **Total of 5 Bt corn varieties for RW control approved by EPA + 5 stacked varieties**
- **Adoption has been rapid, in many areas Bt corn > conventional varieties**
- **Performance has been excellent, very low damage and no documented failures**

# More history of Bt corn

- Thirteen years since first widespread planting of a Bt crop
- No reports of widespread failures (i.e. resistance) to date
- BUT: frequency of resistance genes* in one key pest (corn earworm) has increased substantially
- Suggests that the refuge strategy is working… but likely due to other tactics employed simultaneously

# A brief history of Bt corn

**Bt corn refuge requirements:**

- **20%, but subject to change…**
- **Must be within or adjacent to field for RW hybrids, within ½ mile for ECB hybrids**
- **But… is it *really* necessary?**

# The problem with rootworms: Indiana 2005 emergence data

Assume planting density of 28,000-30,000 plants/acre…

| *Bt* emergence (56 plants)* | Refuge emergence (56 plants)* |
|---|---|
| Males = 135 | Males = 2053 |
| Females = 456 | Females = 2458 |

= approximately 300k beetles/acre of Bt corn

- **WCR**
  - Females don't move before they mate, females mate once
  - Lower-dose toxin (ca. 10% survival), potential for dozens of adults/plant
  - Corn is only N. American host
- **ECB**
  - Both sexes disperse, mate outside cornfields multiple times
  - Higher-dose toxin (< 1% survival), potential for 2-3 adults/plant
  - Many other (non-B.t.) plant hosts in N. America

***All of the above reduce the odds of ECB developing resistance…***

***Why should we worry about WCR?***

# Refuge compliance: Declining

## Report findings:

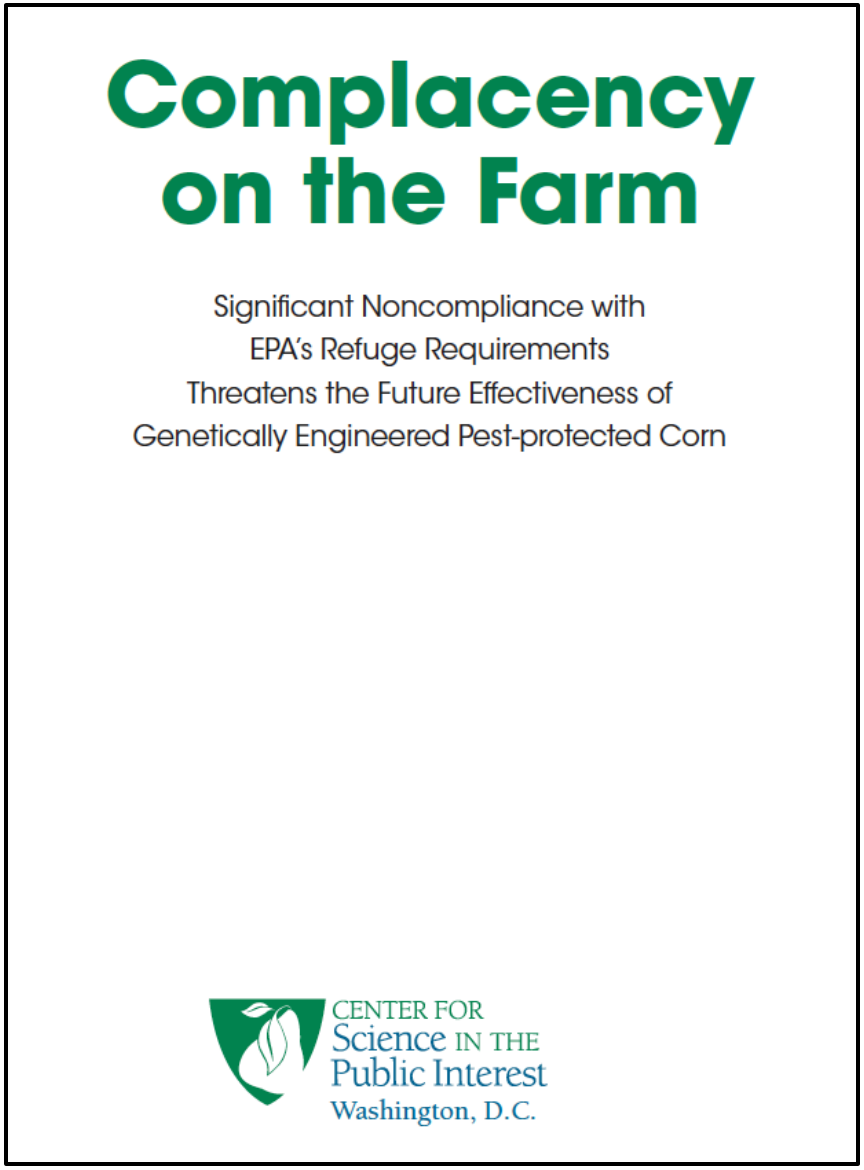
**1.Compliance is dropping – down from 90%+ in 2004 to 66-78% in 2008 (both phone survey and on-farm assessment).**

**2.Non-compliance with *both* size and distance requirements.**

**3.Most non-compliance incidents are "significant deviations"**

***Why?***

**Seems to be correlated with introduction of RW-Bt strains, with more restrictive refuge planting requirements.**



# Improving refuge compliance

**All Bt corn RW registrations expire on September 30, 2010.**

**EPA has requested that registrants increase compliance**

**Possible ways to do this:**

1. **Reduce/simplify refuge options (lower % req'd).**
2. **Make non-compliance impossible (refuge-in-a-bag).**
3. **Guantanamo option (harsher penalties for offenders)**

# *Bt* Corn Registrations for 2010

| Company | Crystalline Protein | Trade Name |
|---|---|---|
| Monsanto | Cry3Bb1<br>Cry1Ab<br>ALL OF ABOVE+RR | ***YieldGard RW***<br>***Yieldgard***<br>***Yieldgard VT Triple*** |
| Dow AgroSciences & Pioneer Hi-Bred | Cry34Ab1 /Cry35Ab1<br>Cry1F<br>ALL OF ABOVE | ***Herculex RW***<br>~~***Herculex I***~~<br>***Herculex XTRA*** |
| Syngenta | mCry3A<br>Cry1ab<br>ALL OF ABOVE | ***Agrisure RW***<br>***Agrisure CB***<br>***Agrisure CB/RW*** |

***Western Bean Cutworm Control***

***ALL the above require planting the 20% non-Bt refuge:**

**- within or adjacent field for RW hybrids**

**- Within ½ mile for CB hybrids**

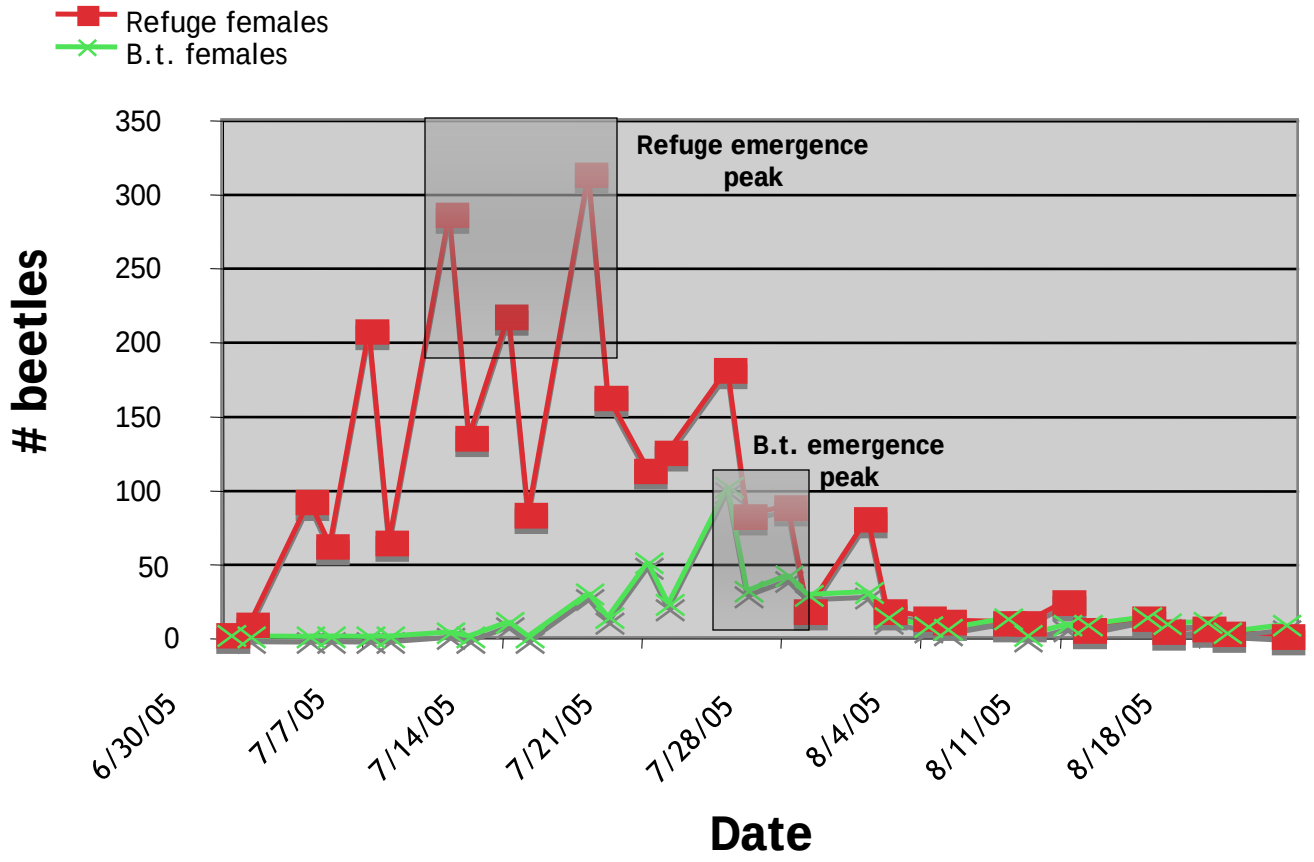
# *Bt* Corn Registrations 2010: New offerings

| Company | Crystalline Proteins | Trade Name | Refuge? | Status for 2010 planting |
|---|---|---|---|---|
| Monsanto+Dow | Cry1F<br>Cry34Ab1<br>Cry35Ab1<br>Cry3Bb1<br>Cry1A.105<br>Cry2Ab<br>RoundupReady<br>LibertyLink | ***SmartStax*** | **5%, must be within or adjacent to field** | **Registered and approved for sale** |
| Pioneer Hi-Bred | Cry34Ab1<br>Cry35Ab1<br>Cry1F | ***Optimum Acremax*** | **Seed is mixed at 2-5%, no additional refuge required** | ***Registration pending, likely to come soon…*** |

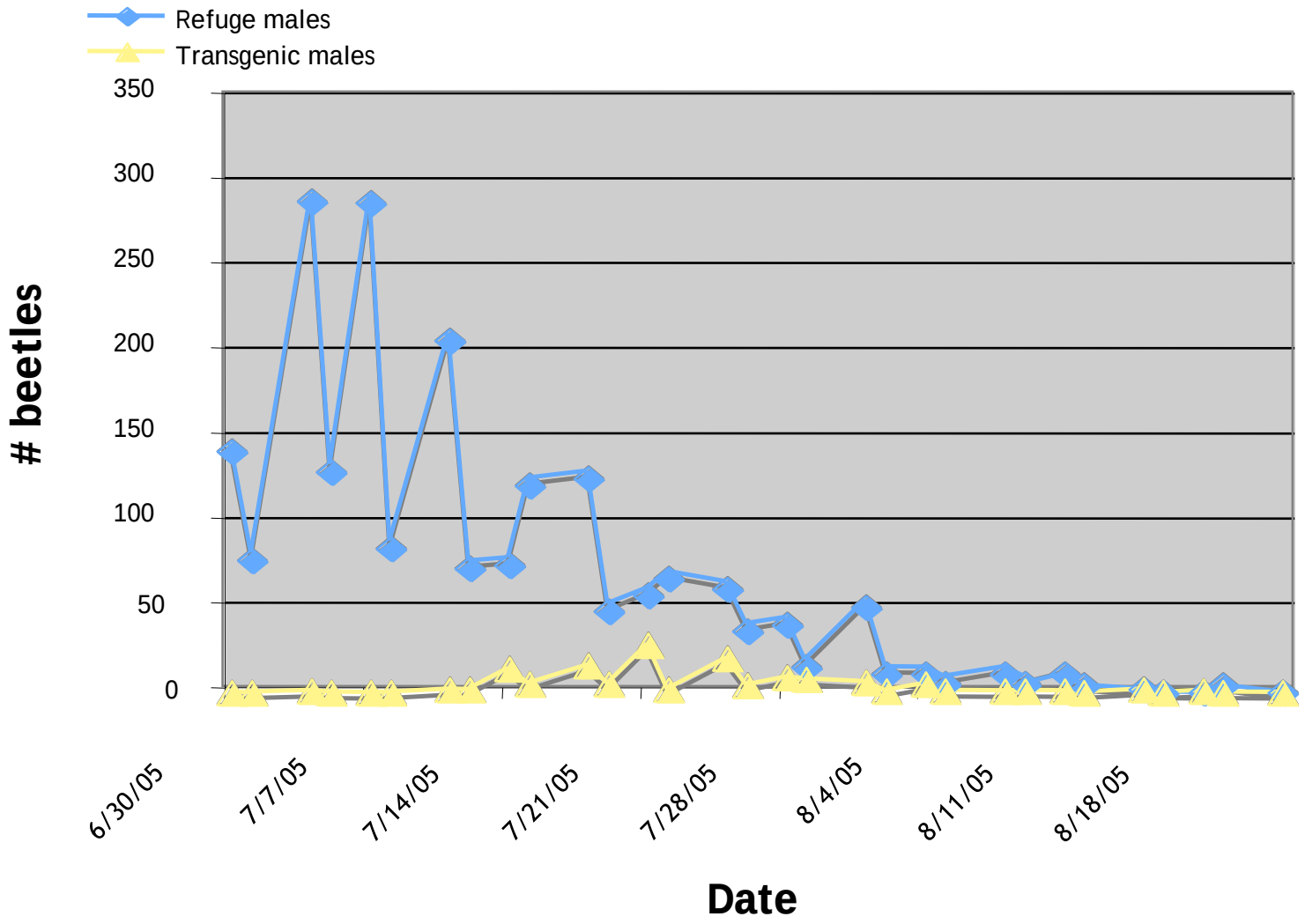
**WBC Control**

# *Recall: Purpose of refuge is to provide susceptible beetles to mate with Bt beetles*

## *Female emergence: Bt vs. refuge corn*

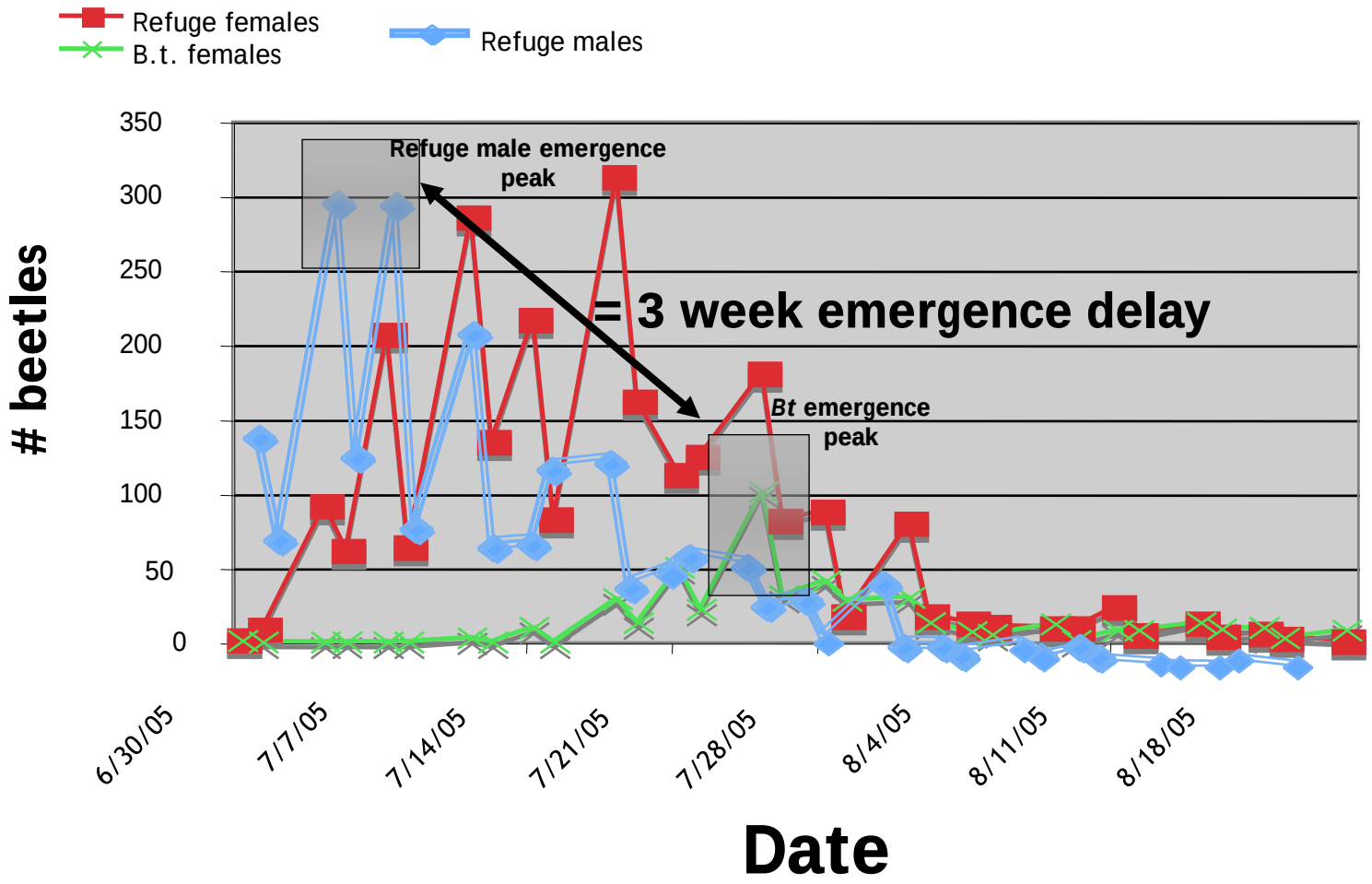


# But…very few males emerge from Bt corn…



# End result:

## *Separation of beetles in space AND time*



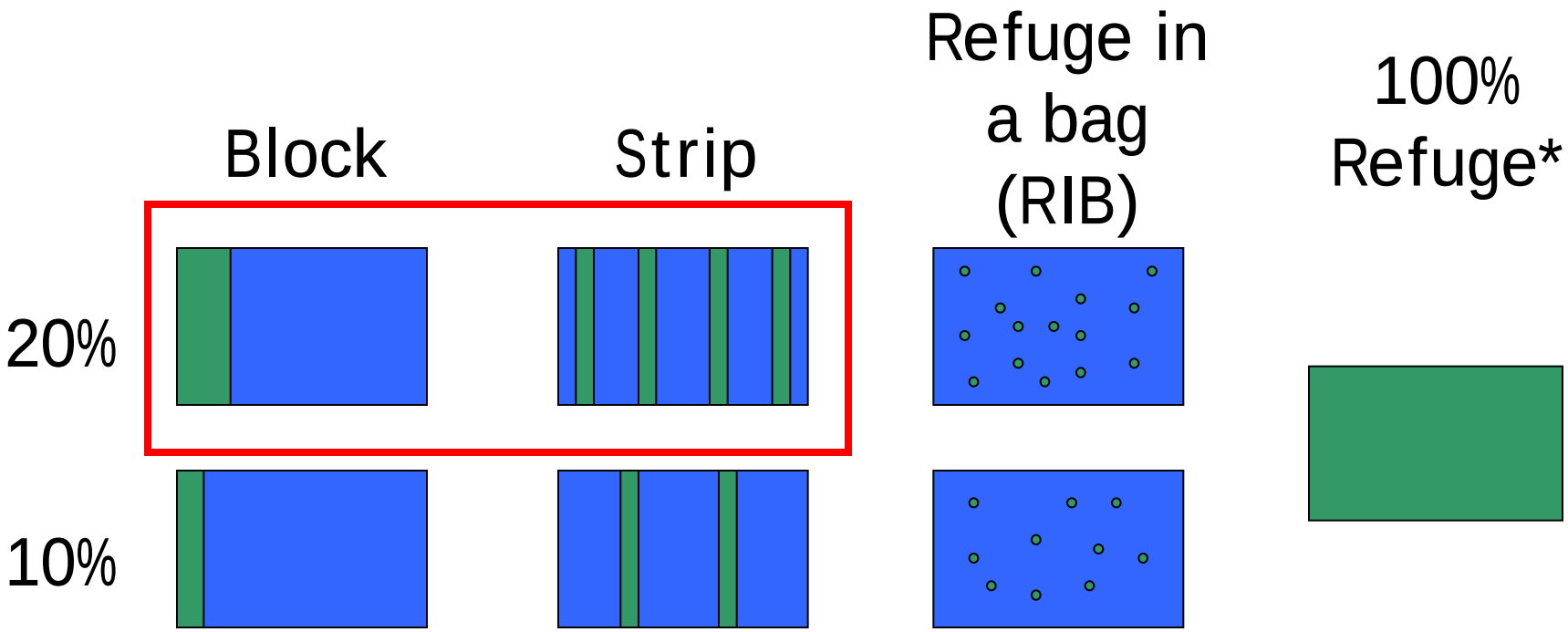
# Evaluating Refuge-in-a-bag

**Objective**:
Document emergence from Bt/refuge plants under varying planting configurations

**Emergence cages:**

- Six per plot
- Checked twice/week
- June 25-Aug 30, 2007 (also in 2008)

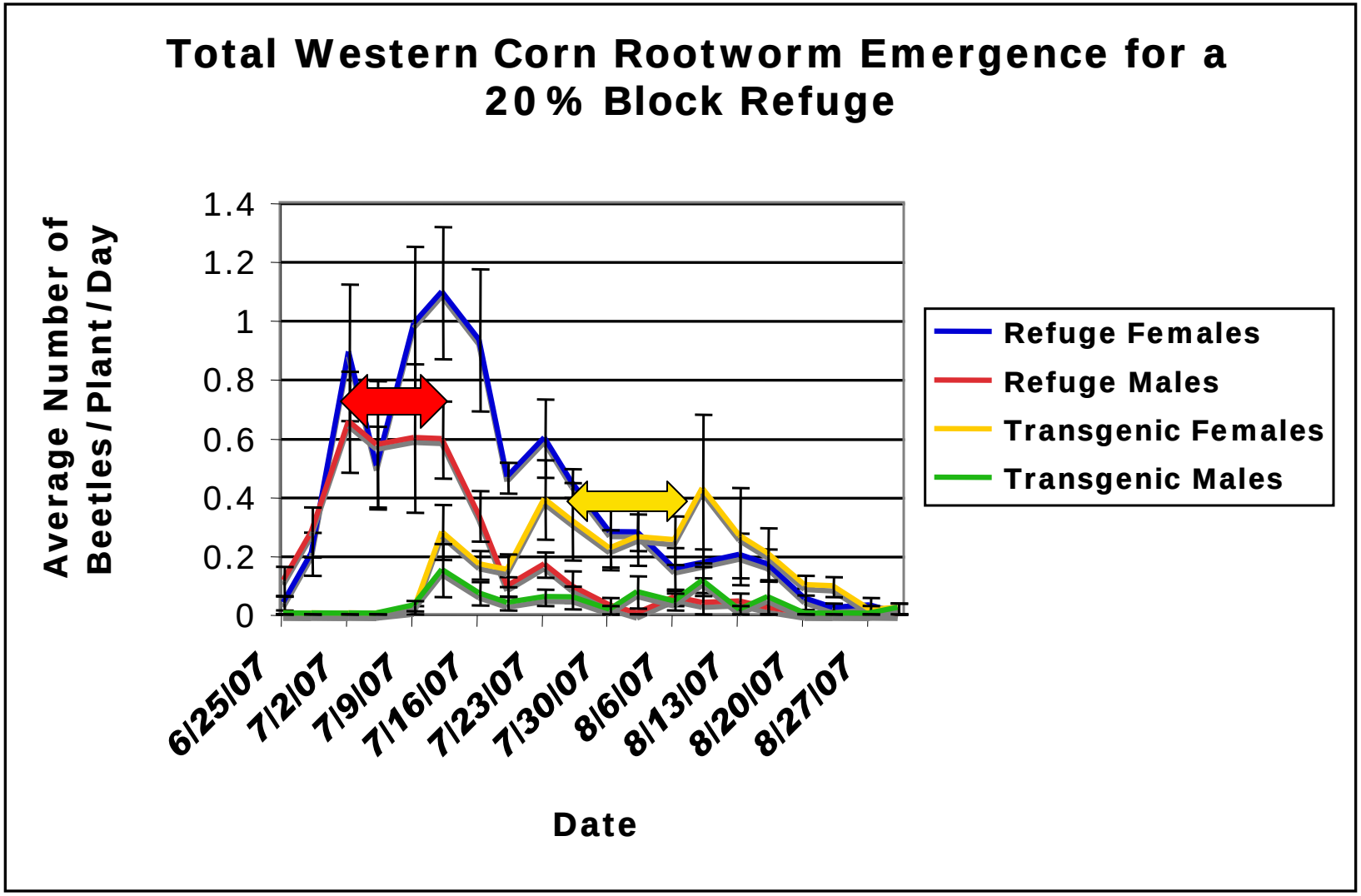
# Evaluating Refuge Structures



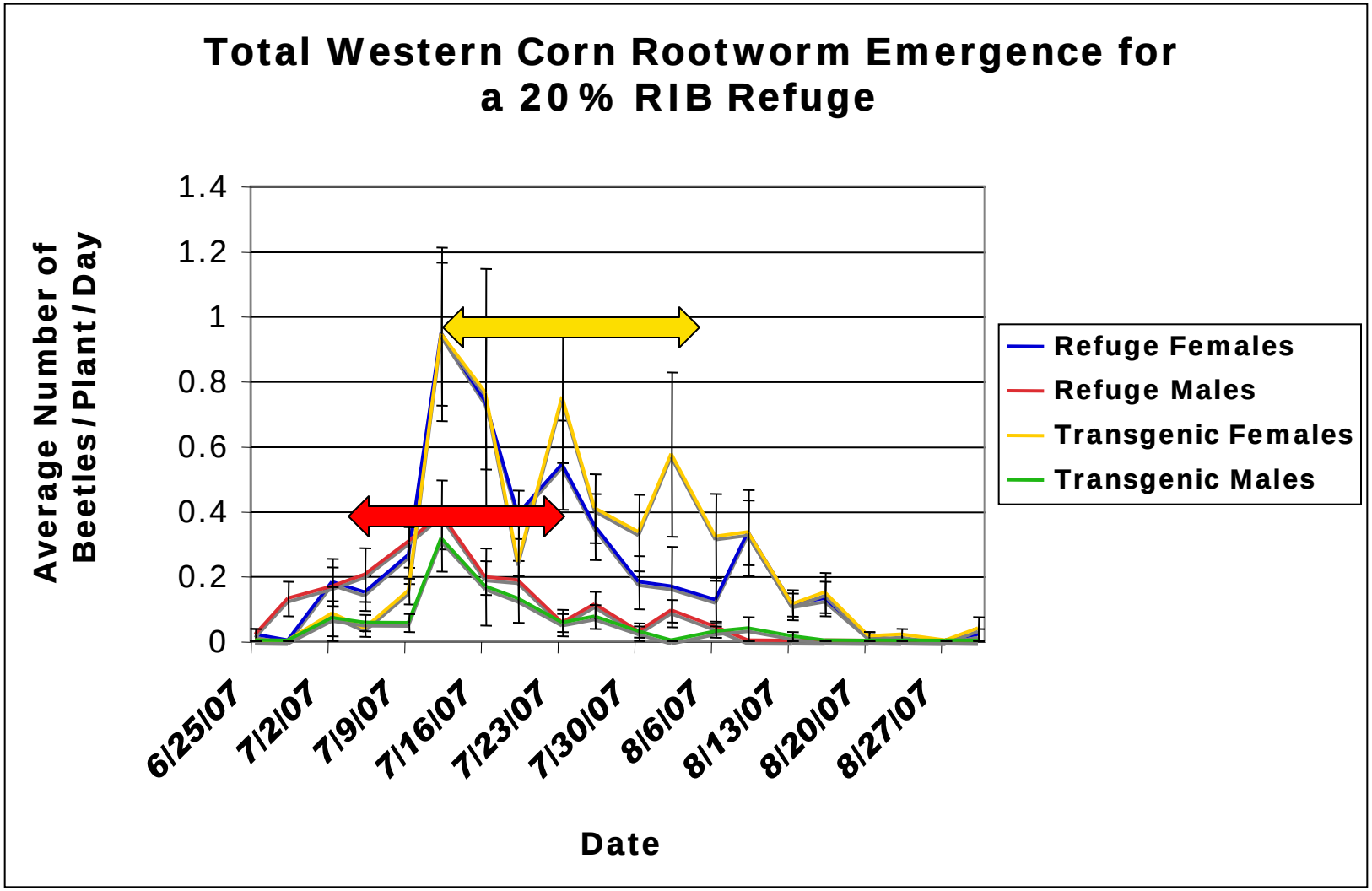

Transgenic

Refuge * Treated with a high rate of clothianidin (Poncho 1250)

# Emergence Trap Results:
# 20% Block

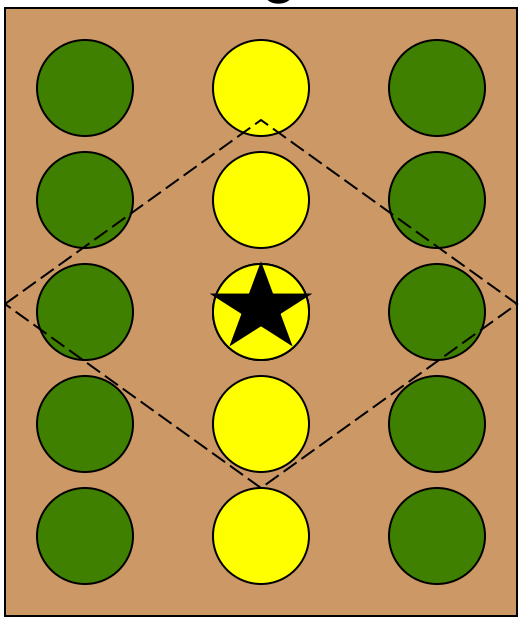


# Emergence Trap Results:
# 20% RIB

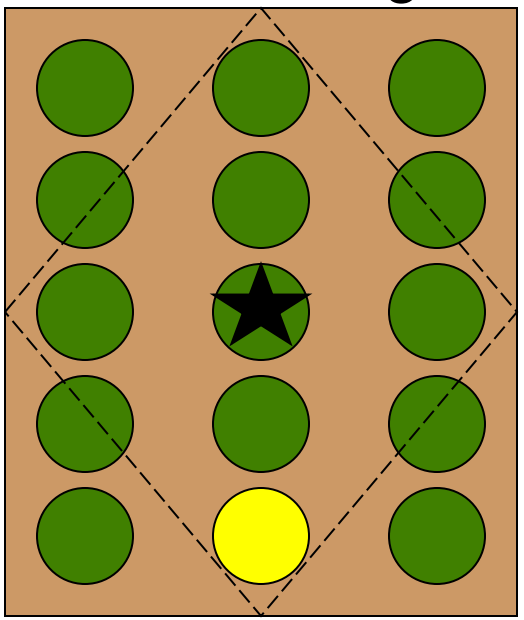


# Effects of neighbouring plants on damage

**Refuge Near Transgenic**

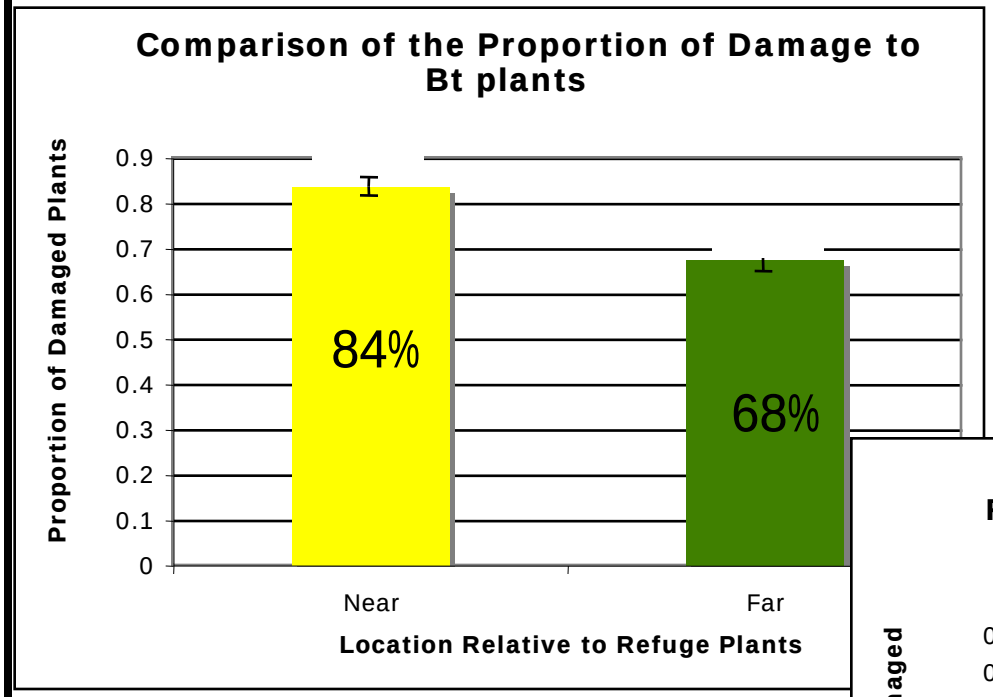


**Transgenic near Refuge**

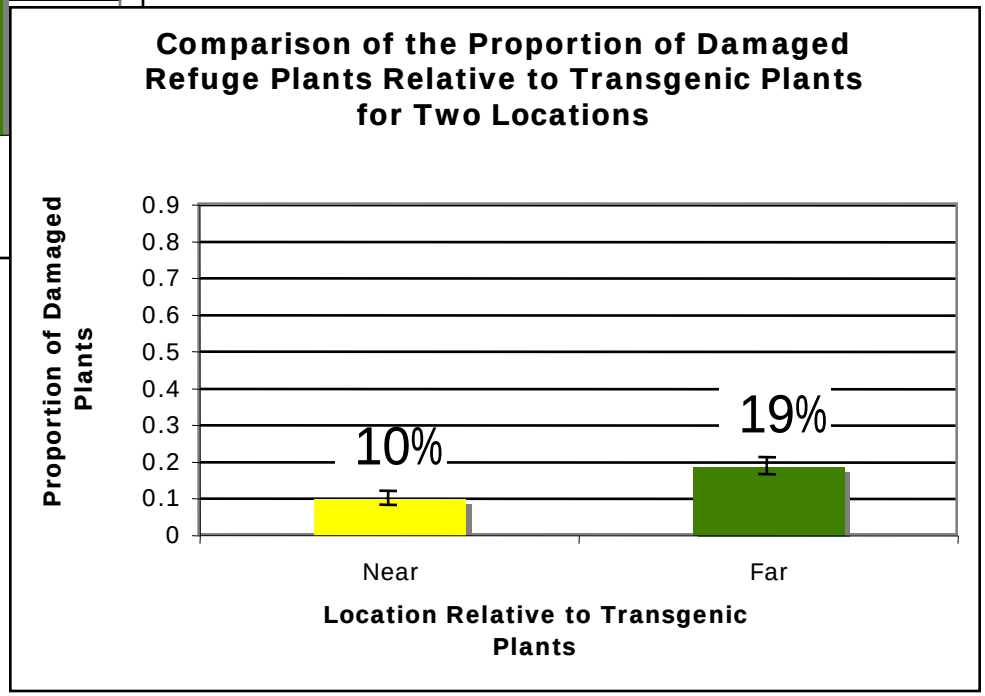


**Refuge** **Transgenic** **Trap**

# Root Rating Results:
# *Bt* plants in seed mixtures




**Significantly more damage on Bt plants located beside refuge ones...**




**...while refuge plants beside Bt sustain less root damage**

***Both indicate larval interplant movement***

# Emergence Trap Results: Summary

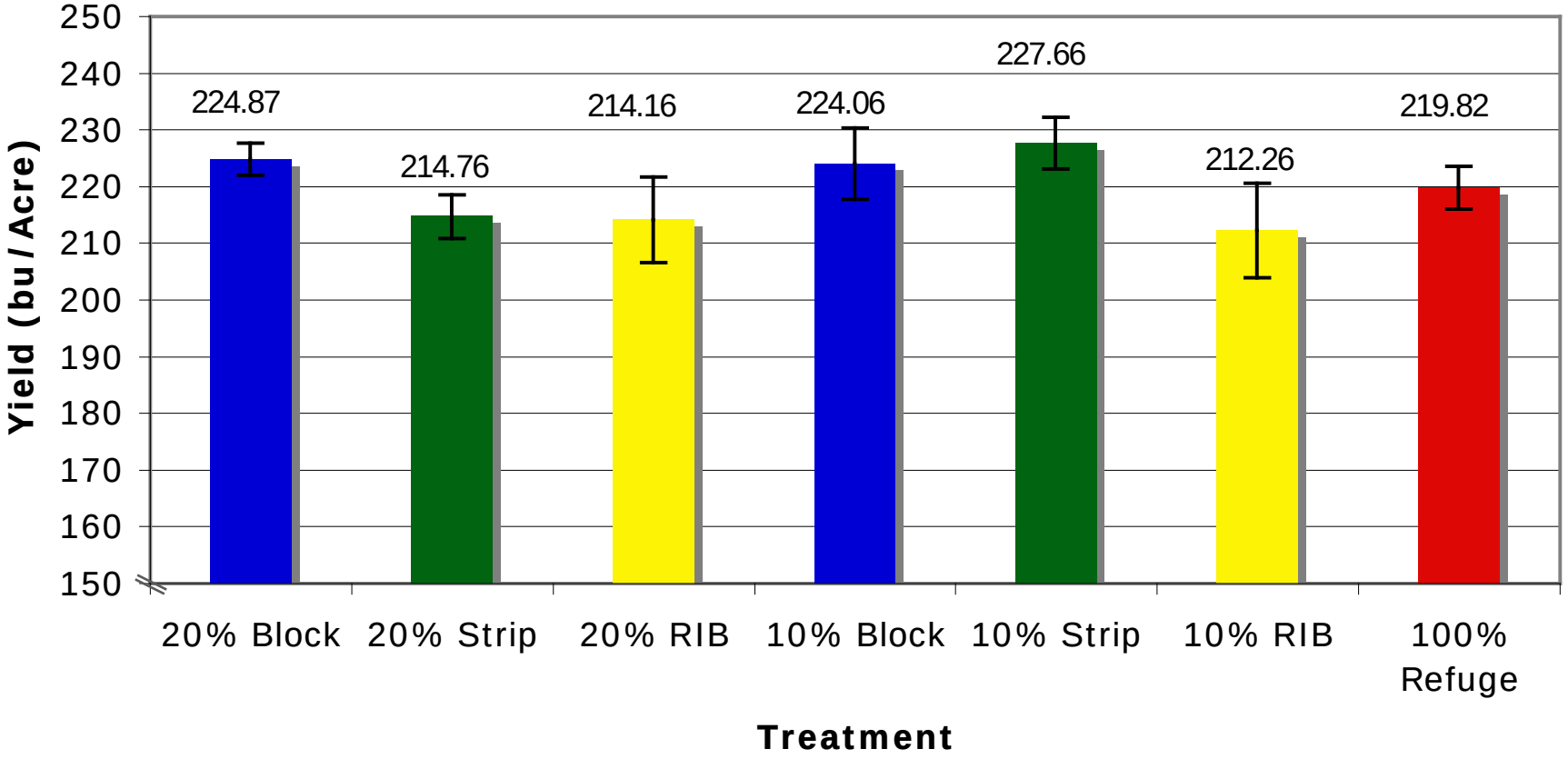
- Using a seed mix causes peaks to synchronize in time and magnitude

- Suggests larval movement, some larvae probably feeding on Bt roots later in life, receiving sub-lethal doses

# Yield

- 5/1000 of an acre/plot
- Hand-harvested in October 2007/08

# Yield Results



# Volunteer Bt Corn: 2007-09 Summary

0.5 VC/m²

2 VC/m²

4 VC/m²

8 VC/m²

12 VC/m²

16 VC/m²

# Volunteer corn yield effects: 2008 soybean yield data

| | kg/ha | Bu/A | p < 0.05 |
|---|---|---|---|
| Untreated | 3555 | 54 | a |
| 0.5 plants/$m^2$ | 3294 | 50 | a |
| 2 plants/$m^2$ | 2796 | 43 | b |
| 4 plants/$m^2$ | 2339 | 36 | c |
| 8 plants/$m^2$ | 2229 | 34 | c |
| 12 plants/$m^2$ | 2017 | 31 | c |
| 16 plants/$m^2$ | 2039 | 31 | c |

# Volunteer Corn in Soybeans: 2007 Methods and results

- 8 fields surveyed in June/July 2007
- All planted with Yieldgard VT ("triple-stack") in 2006, RR soybeans in 2007
- 732 volunteer corn plants tested for Bt/RR and rated

1. ***Volunteer corn is hard to kill with glyphosate…***
   **87% of volunteer plants possessed the RR gene**

2. ***Volunteer corn may be "Bt corn"***
   **64% of volunteer plants expressed Bt-RW protein**

3. ***Volunteer Bt-corn may not express protein at optimal rates:***
   **Some severe feeding damage on Bt-RW roots…**

# Volunteer *Bt* Corn in Soybeans: 2007 Results

| Damage category | % with Bt |
|---|---|
| No damage  | 37% |
| Mild (≤ ½ node)  | 35% |
| Severe (> ½ node)  | 28% |

# Volunteer *Bt* Corn in Soybeans: 2007 Results

| Damage category | % with Bt | % without Bt |
|---|---|---|
| No damage  | 37% | 32% |
| Mild (≤ ½ node)  | 35% | 33% |
| Severe (> ½ node)  | 28% | 34% |

# *Do beetles emerge from Bt+ VC in soybeans?*

- **WCR emergence traps**
  - **1 trap per 0.5 and 16 plant/m$^2$ plot (Bt+ VC)**
  - **4 control traps (Bt negative plants)**
  - **28 traps per site location**
  - **2 site locations**
- **Collected adult beetles weekly**

# Beetle emergence - Results

## 2008 WCR emergence data

| Trap Type | Mean # WCR/trap (SE) |
|---|---|
| **16 plants/m$^2$** | 2.45 a |
| **0.5 plants/m$^2$** | 0.74 b |
| **Control (Bt negative)** | 0.65 b |

# Volunteer *Bt* Corn in Soybeans

**Q: Why so much damage/emergence from volunteer Bt corn?**

1 ~~Intense pressure – no other hosts to feed on~~

2 Lower Bt toxin expression

**Unlikely. Damage on Bt- VC plants should be higher if this is the case**

# Volunteer *Bt* corn in soybeans: How toxic is it?

**Toxin dose experiments**

**Use protein test strips to test 1 cm sections of VC roots**

***vs.***

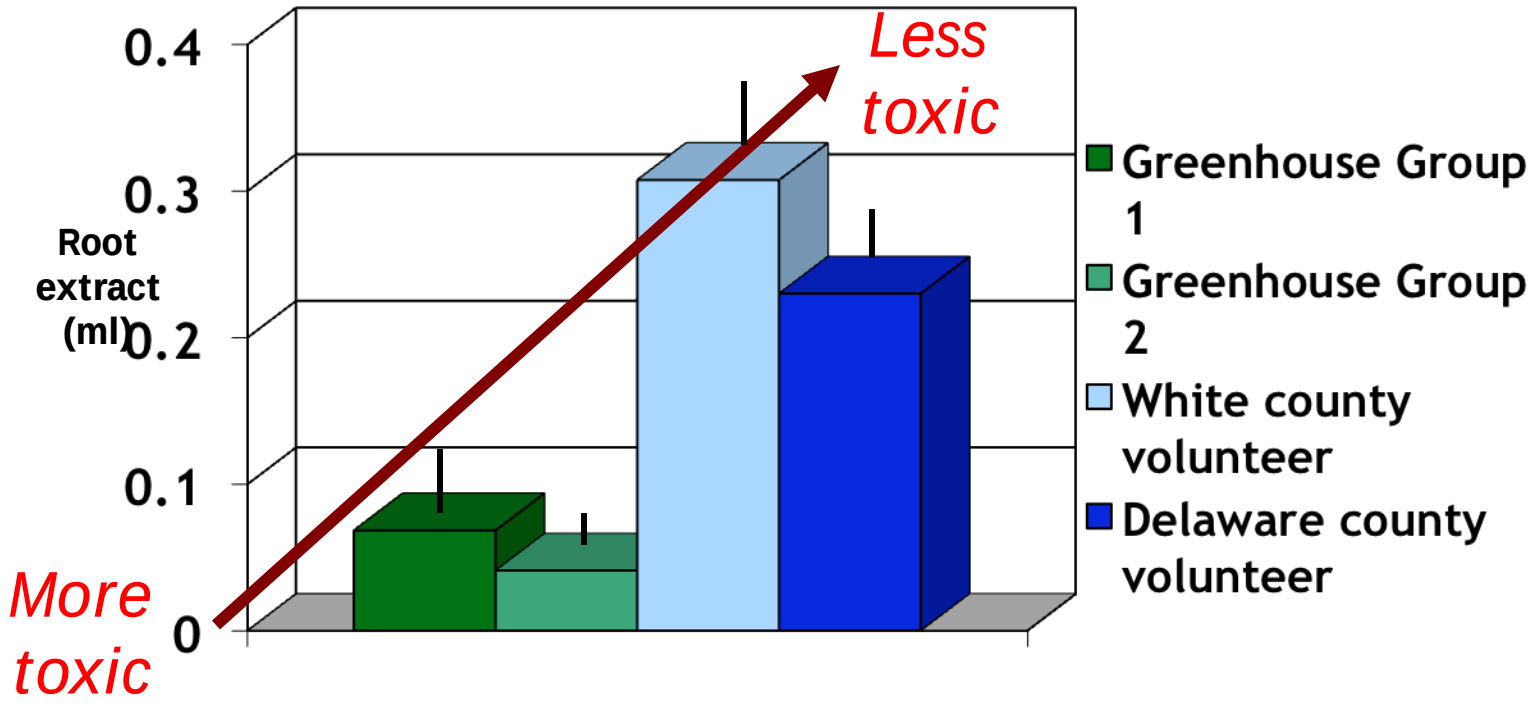
**Greenhouse-grown, commercial Bt roots (DKC 61-69)**


**Progressively diluted starting stock to determine minimum required for positive test**

***i.e. Higher Bt content in tissues = less initial material for positive test***

# Volunteer *Bt* Corn in Soybeans: 2008 Results

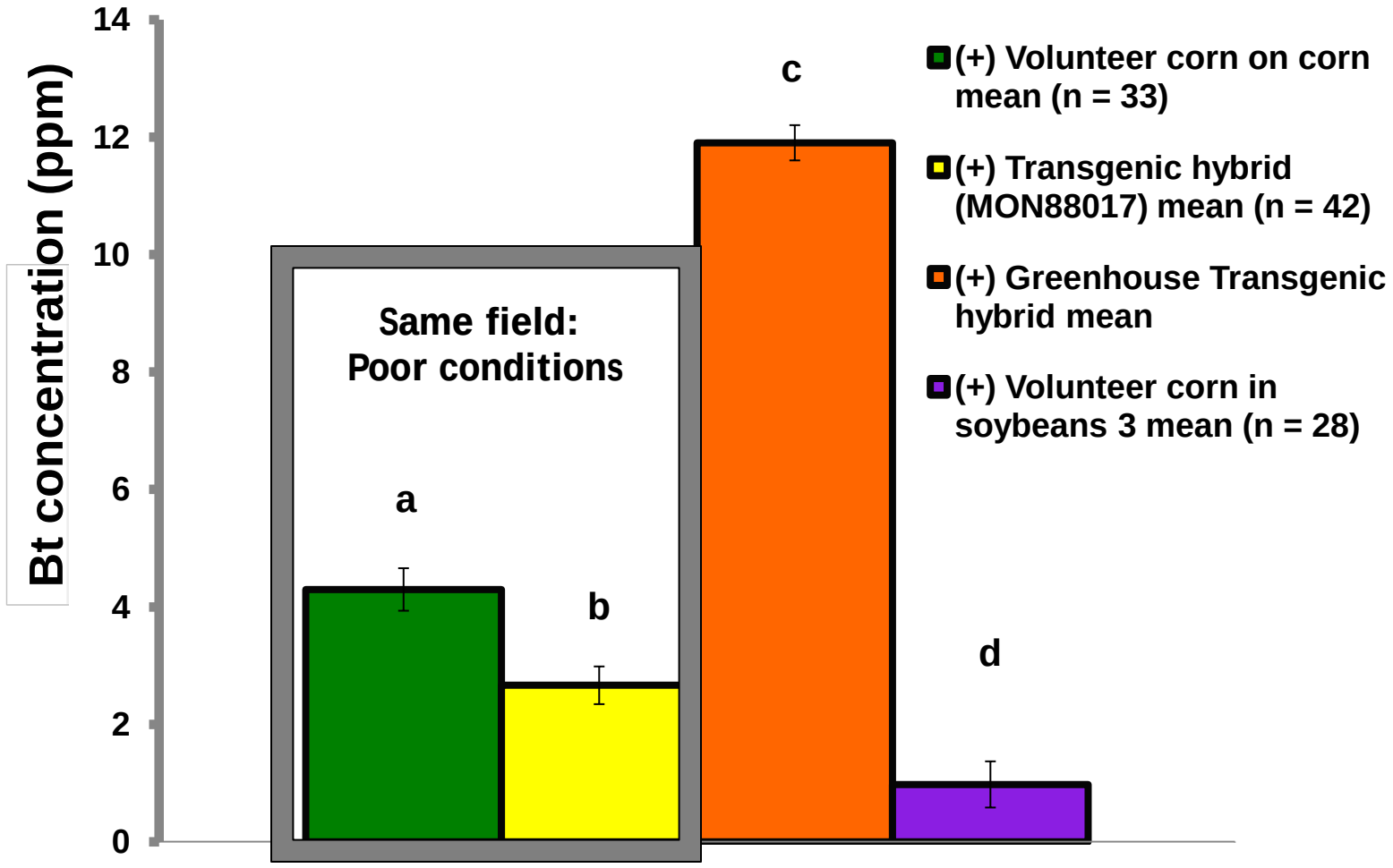
*Minimum volume of root extract to generate positive test*



# Volunteer *Bt* Corn: Other scenarios

- **2009: Partnered with Monsanto to develop more detailed Bt VC concentration data**
- **4 treatments: VC in soybeans, VC in corn, Bt hybrid (field), Bt hybrid (greenhouse)**
- **Removed 1 cm section of new, growing root**
- **Shipped to Monsanto for ELISA test**
  **- all samples “blind”**

# Volunteer Corn: Bt expression

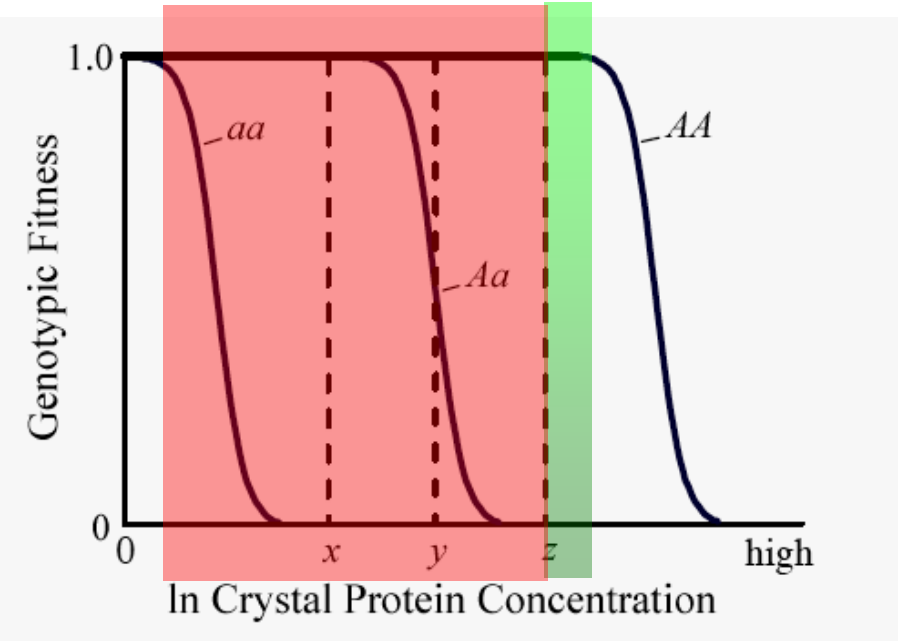


# Volunteer *Bt* corn in soybeans: Why is it important?

There will *always* be many RW eggs in fields following corn…

If *Bt* dose is <u>not</u> maximum, more weakly-resistant larvae can survive exposure and develop on “islands of resistance”.

Potentially much more of an issue in continuous corn.



# Volunteer *Bt* Corn in Soybeans: 2010+ recommendations

As herbicide-tolerant corn becomes the norm, incidence of volunteer corn will increase*, RW larvae can complete development upon Bt+ VC plants

Solution:
Treatment with grass herbicide *early in season* (pre-June 15) will prevent RW survival

# SmartStax Thoughts/Observations

Combines the three major Bt toxins vs RW in one – modes of action are supposedly independent, this allows reduction of refuge to 5%

However, emergence data for the combo are higher than expected… Suggests that beetles have some way of surviving both that is not understood.

How do beetles survive Bt exposure? Still unknown… could be physiological, behavioral (probably both)

Stacking toxins is the shape of things to come – until somebody finds a high-dose toxin to put into plants vs. WCR

# Final Thoughts/Summary

New Bt corn offerings largely driven by need to increase refuge compliance

Refuge-in-a-bag options offers benefits, but increases sub-lethal exposure + risk of damage to Bt plants

Volunteer corn incidence will probably continue to increase, but manageable in soybeans (more problems in continuous corn?)

Volunteer corn following Bt corn may represent "islands of resistance" – kill it!

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